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October 4, 2019

RE: Resubmission of the manuscript titled "*A data integration workflow to identify drug combinations targeting synthetic lethal interactions*" JoVE60328 - [EMID: a39e91c34e87e70e]

Editorial and Reviewer Team
JoVE

Dear Editors and Reviewers,

Thank you for the opportunity to revise our manuscript titled "*A data integration workflow to identify drug combinations targeting synthetic lethal interactions*". This letter includes a line-by-line response to each of your valuable and constructive comment; highlighted in bold to improve readability. We believe that our manuscript has substantially improved after making the suggested changes.

Sincerely,

Univ.-Prof. Dr. Michael Krainer

Editorial Comments

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.
We have proofread the manuscript and corrected all the typographical errors to the best of our knowledge.

• Protocol Language: The JoVE protocol should be almost entirely composed of numbered short steps (2-3 related actions each) written in the imperative voice/tense (as if you are telling someone how to do the technique, i.e. "Do this", "Measure that" etc.). Any text that cannot be written in the imperative tense may be added as a brief “Note” at the end of the step (please limit notes). Please re-write your ENTIRE protocol section accordingly. Descriptive sections of the protocol can be moved to Representative Results or Discussion. The JoVE protocol should be a set of instructions rather a report of a study. Any reporting should be moved into the representative results.
1) Examples NOT in imperative voice: step 1.1, 2.1, 3.1, etc
We rewrote the Protocol section in imperative voice (with the exception of Notes).

2) For code snippets, please add a step before each code block describing the actions.
We have added a comment line before each code block summarizing the actions.

3) Please remove the enclosing boxes around the code blocks.
The enclosing boxes around the code blocks have been removed

4) Split up long steps (e.g., 2.1) into 2 or more steps.
We have tried our best to split long sentences for purposes of readability.In 2.1, we have omitted the example query and displayed only show the final data retrieval step.

• Protocol Detail: 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 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.
All commands as well as links for downloading data in order to execute the workflow have been provided in the form of code snippets.

• Protocol Highlight: Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE’s instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.
1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.

2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
4) Notes cannot be filmed and should be excluded from highlighting.
We have highlighted the relevant steps in yellow as suggested.

• Discussion: JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.
We currently have 5 paragraphs in the Discussion section elucidating the overall aim of the workflow, modifications and extensions along with notes on troubleshooting, limitations and potential modifications and filtering steps to overcome the limitations, as well as critical steps. Thus, we believe that the Discussion section sufficiently follows the guidelines of the journal.

• Tables: Please remove the embedded Table from the manuscript. All tables should be uploaded to the Editorial Manager site in the form of Excel files. A description of the table should be included with the Figure legends.

We have removed the embedded Table and added the Table legend to the Figure legends section. The Tables are now provided as separate Excel files.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."
Please see attached E-mail permissions to re-use the figures from previous publishers.

<u>Reviewers' Comments</u>

Reviewer #1
Manuscript Summary:
The manuscript proposes a workflow that integrates information from different sources to reveal potential drug combinations targeting synthetic lethal interactions, It provides a couple of previously reported examples of its applications. It is well-written; the protocol is crystal clear. Just minor comments from my side.

Minor Concerns:
* Could you please elaborate on the following statement "Relevance of organisms for the subsequent

translation step to human genes can be identified by counting the number of occurrences of organism tax-IDs". Please provide references.
The statement has been rewritten as follows: “Determine the number of synthetic lethal interaction partner tax-IDs to get an estimate on the number of synthetic lethal interactions being available per organism”.

* The authors say that, when testing drug combinations, it must be ensured that neither of the two drugs alone is targeting both synthetic lethal interaction partners. This is reasonable for the sole purpose of a combined therapy approach but if one of the retrieved drugs targets both targets simultaneously, it could be a valuable multi-target agent. Maybe a note on this would be appreciated.

The following Note has been added for purposes of clarity: “A drug that would target both synthetic lethal interaction pathways would be toxic to any cell, so theoretically it is not a valuable multi- target agent. That is the reason why this possibility is excluded in this step of the algorithm.”

* Please specify which folds may be advised in "Dilute drugs in solvents such as DMSO or phosphate-buffered saline and us them in combination or alone for treatment of cells at folds of their previously established IC50 (inhibitory concentration)."
We have edited the above sentence for purposes of clarity: "Dilute drugs in solvents such as DMSO or phosphate-buffered saline in at least four different concentrations based on their previously established IC50 inhibitory concentration and use them in combination or alone for treatment of cells.”

* Why have the authors tested such high levels of celecoxib and zoledronic acid in their combination experiments? As the authors themselves note, such levels would be hardly met in a therapeutic setting.
We have added the following sentence in the Discussion section for purposes of clarity: “The concentrations were chosen based on cell culture experiments with these given drugs in the literature.”

* By the way, I think "supratherapeutical" is better than "supraphysiological", since, as xenobiotics, the idea of a physiological concentration does not make sense.
We have substituted “supraphysiological” with “supratherapeuticel” as suggested by the reviewer.

There are minor typos.
We have proofread the manuscript and corrected all the typographical errors to the best of our knowledge.

Reviewer #2
Manuscript Summary:
The manuscript describes a protocol for extracting synthetically lethal pairs of genes from BioGrid, mapping the pairs to their human orthologues, and then integrating data from DrugBank and clinicaltrials.gov to identify druggable protein pairs.

These protocols are straightforward and could be successfully implemented by novice bioinformaticians -which would be helpful to the community.

Major Concerns:
i) My major issue is the underlying concept.
While is is true that if you simultaneously drug 2 synthetically lethal proteins you have the ability to kill a cell, this would be of little practical use as this would affect healthy cells as well as cancer cells.
The therapeutic attractiveness of synthetic lethality is when one protein is genetically inactivated in the tumour, ie is an inactivated tumour suppressor - BRCA1/2 in the clinic, and the other is inactivated pharmacologically - PARP1.
In healthy cells, BRAC1/2 work, so even though PARP1 in inhibited the cells can function.
This gives the therapeutic rationale (ie kills cancer cells but leaves healthy cells viable). This was not explained in the paper.
The example that they give is where they attack 2 proteins in a SSL pair; this would also be toxic in healthy cells.
The experimental confirmation although valid- they need a control. i.e it needs to be tested in a healthy cell as well as a cancer cell line. Most likely it will kill the healthy cell so is of no use as a drug treatment.
The concept does not directly result in ideal therapeutic combinations useful for cancer therapy. However, it can predict lethal interactions of non-toxic drugs based on synthetic lethality in cell culture. Consequently, in further experiments non-toxic drugs can be applied to cancer cells with defined genetic deficiencies in the affected pathway and a therapeutic index can be expected.

ii) The prediction method. Although there is certainly an enrichment of pairs of proteins synthetically lethal in humans that are synthetically lethal in model organisms, the actual prediction accuracy is very low. The authors should have produced a ROC curve to show the performance of their algorithm.
We agree with the reviewer that the prediction performance is of interest to the reader. Unfortunately, we feel that this would be out of the scope of this manuscript; however, reference 6 in reference 25 of this manuscript provides further details on the prediction performance.

iii) Finally, the genetic interaction data itself can be quite inaccurate. For model organisms where a lot of data is available, multiple observations improve the reliability of the data.
We are in full agreement with the reviewer and have added the following text in the Discussion section: "One might consider restricting the input set for the orthology mapping to data from mouse and rat only, which are evolutionary closer to humans. An additional way of defining the input set of synthetic lethal interactions is to only focus on synthetic lethal interactions being conserved in multiple species, thereby increasing the chances that the synthetic lethal interaction is truly positive."

Reviewer #3
Manuscript Summary:
The manuscript describes a work on identifying drug combinations that can target synthetic lethal proteins, so that in cancers, for instance, where the absence or mutation of one protein exists, the other protein can be a good candidate drug target to stop the proliferation. A protocol to identify such drug combinations is proposed. It is well written and easy to follow. I think it will be of great interest to researchers in this field.

Major Concerns:
None.

Minor Concerns:
My only minor concern is the quality of the figures. It is not of high quality in the current manuscript. It will increase the readability if the authors provide higher quality figures.
We have uploaded figures in high-quality for production.

Reviewer #4
Manuscript Summary:
This manuscript provides a protocol on how to generate a list of drugs through synthetic lethality using bioinformatics. The authors provided a workflow and the codes on the protocol, and showed the results in their studies. Overall, the manuscript is well written, and the steps are clear. However, the authors need to tidy up their codes, specifically providing more annotations/comments in their codes to show users what those values mean (e.g. column numbers, taxa etc).

Major Concerns:
1. In the codes, please make annotations for ease of understanding. For example, in pg9, code starting from line 198: what is the "559292" means in the "print 559292" ? is it the number of interactions? Or what? If this is a fix value, it should be defined properly. For example, the same code, the "9606" in the line if($5 == 9606), this reviewer understands that this is the taxa code for human. This should be defined as a variable "$TAXA = 9606"; and in the line (if $5 == $TAXA). Then user will know how to change the value "$TAXA" to other species. Similar cases exist in other places of various codes.
We thank the reviewer for this comment. As suggested, we have used variables for tax ids to avoid magic numbers in the code. We also agree with the reviewer that further constant values (only string constants e.g. Negative Genetic or Humans remaining) could be factored out into variables; however, we think that this would only complicate the code as the constant value is equally meaning full as any variable name that could be used instead. Therefore, we prefer to keep them directly in the code.

2. The authors assumed the users/readers will know the columns name. For example: in page 11, the authors are extracting some columns from DrugBank csvtool col 3,12,13 -u TAB "${DB_TARGETS}. The authors should do the annotation for these columns similar to Table 1 for BioGrid. This will inform the users, and potentially DrugBank might change their columns in the future, and the users still know which column to extract from.
Thank you for this valuable comment. The suggested Table 2 has been included.

Reviewer #5
Manuscript Summary:
The authors presented an integrated workflow for identification of drug targets for synthetic lethal interacting pairs against ovarian and breast cancer exploiting the information available in already existing databases. Their workflow provides a comprehensive step by step procedure which can easily be replicated. The authors have systematically applied the current protocol to two already published studies for analyzing drug combinations currently in clinical use or investigated in late stage phase III or IV clinical trials against ovarian and breast cancer. Additionally, they have proposed other drug combinations which are not being tested in clinical trials but exhibit a synthetic lethal mechanism of action for cancer. Overall, the paper is reporting valuable information on possibility of selectively targeting cancer cells, with the potential to reduce drug resistance and side effects. Even though only

one synthetically lethal interaction - between PARP and breast-cancer BRCA1 and BRCA2 is clinically approved, nevertheless such interactions have potential for clinical applications.

The article may be accepted in its current form.