Chemogenetic profiling reveals PP2A-independent cytotoxicity of the proposed PP2A activators iHAP1 and DT-061

Gianmatteo Vit, Joana Duro, Girish Rajendraprasad, Emil Hertz, Lay Katrine Holland, Melanie Weisser, Brennan McEwan, Blanca Lopez-Mendez, Paula Sotelo-Parilla, A. Arockia Jeyaprakash, Guillermo Montoya, Niels Mailand, Kenji Maeda, Arminja Kettenbach, Marin Barisic, and JakobNilsson

DOI: 10.15252/embj.2022110611

Corresponding author(s): Jakob Nilsson (jakob.nilsson@cpr.ku.dk)

Review Timeline:

| Event                | Date       |
|----------------------|------------|
| Submission Date      | 7th Jan 22 |
| Editorial Decision   | 10th Feb 22|
| Revision Received    | 6th May 22 |
| Editorial Decision   | 16th May 22|
| Revision Received    | 18th May 22|
| Accepted             | 19th May 22|

Editor: Hartmut Vodermaier

Transaction Report

Please note that the manuscript was previously reviewed at another journal and the reports were taken into account in the decision making process at The EMBO Journal. Since the original reviews are not subject to EMBO's transparent review process policy, the reports and author response cannot be published.
Thank you again for transferring your manuscript together with referee reports from a another journal to The EMBO Journal. Following our own assessment of the study and your responses to the previous reviews, as well as discussions with expert editorial advisors, we conclude that a study revised along the lines suggested in your tentative response letter would be of interest to our readership. I am therefore inviting you to resubmit a new version incorporating the already obtained data and planned experiments, as well the various other clarifications. In particular, it should be good to include results obtained with the mentioned new antibody for holoenzyme immunoprecipitation. In addition, please rewrite particularly the abstract and extend the background introduction as appropriate for a stand-alone publication. Please do not hesitate to contact me in order to discuss any specific points ahead of resubmission.

Detailed information on preparing, formatting and uploading a revised manuscript can be found below and in our Guide to Authors - adhering to these guidelines as closely as possible should greatly facilitate editorial processing at the resubmission stage.
Major new experimental data added to the revised manuscript.

We thank the reviewers for the comments provided to improve our manuscript. We have added the following major new data to our manuscript to strengthen our arguments and address concerns raised by the reviewers:

1) We have analysed if perphenazine (PPZ) interacts with PPP2R1A as originally claimed in Gutierrez et al., 2014. This is important to establish, as PPZ is the parent compound providing the starting point for iHAP1 development and mode of action of DT-061. The fact that PPZ can directly bind to PPP2R1A was newer shown by Gutierrez et al. (the authors just referred to a personal communication). Using ITC and NMR, we could not detect any binding of PPZ to PPP2R1A and we see no effect of PPZ in enzymatic assays (new data in Fig. 1, EV1 and Appendix S1-3). Our data thus question the mode of action of PPZ as claimed by Gutierrez et al. This is in line with our inability to detect direct effects of iHAP1 and DT-061 on PP2A complexes.

2) We have analysed the effect of DT-061 on stabilizing reconstituted PP2A-B56α using mass photometry (MP) measurements. Using the same concentrations of holoenzyme as in Leonard et al., we see no effect of DT-061 (new data in Fig. 1D).

3) We analysed the effect of DT-061 on the endogenous holoenzymes in HeLa and H358 cell lines using size exclusion chromatography of cell extracts, since we could not identify an antibody able to immunoprecipitate endogenous PPP2R1A. Using this approach, we see a clear co-migration of endogenous B56α with PPP2R1A and no sign of free B56α, which would argue that there is no free B56α which DT-061 can act on. Furthermore, addition of DT-061 to cells and cell extract did not result in an increase in B56α co-migrating with PPP2R1A (new data in Appendix Fig. S6-7).

4) We have used an affinity tagged form of PPP2R1A to take a similar approach as in Morita et al. and Leonard et al. However, we did not detect an effect of PP2A-B56 composition by addition of DT-061 or iHAP1 (new data in Fig. 2C).

5) In an attempt to “mimic” the proposed mechanism of action of DT-061, we have used a stable cell line expressing inducible YFP-B56α. We have previously shown that this YFP-B56α is functional, as it can suppress RNAi depletion of all B56 isoforms. After inducing YFP-B56α expression, we see a large increase of PP2A-B56α holoenzyme formation by size-exclusion chromatography, yet no effect on cell growth. Under similar conditions DT-061 blocked cell growth (new data Fig. EV2B-C).

6) We have expanded our analysis of the cryo-EM structure reported in Leonard et al. and compared the tail of PP2AC to that of previous structures. All previous structures support the conclusion that the assignment of DT-061 is unambiguous and can also be attributed to residues from the PP2AC tail (new analysis in Fig. EV2 and Appendix Fig. S8).

7) We show that the effect of iHAP1 on microtubules is not prevented by okadaic acid (we use it at concentrations that fully inactivate all PP2A complexes). This argues that the effect is not mediated by PP2A complexes (new data in Fig. 4).

8) We have expanded our analysis of the effect of DT-061 on ER and Golgi markers to H358 cells and have quantified all experiments. We have furthermore included okadaic acid treatments to determine if the effects we see with DT-061 are dependent on PP2A activity. The overall conclusion is that DT-061 affects Golgi and ER markers in both cell lines and largely independently of PP2A activity (new data in Fig. 6-7).
Thank you for submitting your revised manuscript to The EMBO Journal. I have now carefully gone through your responses to the transferred original referee reports, and the changes made in response to my original decision letter. I am happy to say that we can now offer publication in our journal, as soon as a few remaining editorial points listed below have been addressed:

2nd Authors’ Response to Reviewers

18th May 2022

The authors have made all requested editorial changes.
Thank you for submitting your final revised manuscript for our consideration. I am pleased to inform you that we have now accepted it for publication in The EMBO Journal.
Experimental study design and statistics

The data shown in figures should satisfy the following conditions:

- The data were obtained and processed according to the field’s best-practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- Tests and method(s) used to carry out the reported observations and measurements are described.
- An explicit mention of the statistical and chemical entity(ies) that are being measured.
- The exact sample size (n) for each experimental group/condition, given as a number; not a range.
- A description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- A statement of how many times the experiment shown was independently replicated in the laboratory.

Definitions of statistical methods and measures

- Common tests, such as t-tests (please specify whether paired or unpaired), simple t-tests, Wilcoxon, and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section.
- Error bars, one-sided or two-sided?
- Are there adjustments for multiple comparisons?
- Exact statistical test results, e.g., t values = ±s, or p values = ±s.
- Definition of ‘center values’, as median or average.
- Definition of error bars as±s.d. or ±s.e.m.

Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data Presentation.

Materials

- Study design and statistics

- Study protocol

- Study protocol has been peer reviewed, provided DOI in the manuscript. For clinical trials, provide the trial registration number (NIH ClinicalTrials.gov or WHO ICTRP), where applicable.

- Laboratory protocol

- Provide all other data collection details, if external detailed step-by-step protocols are available.
Reporting

Ethics

Sample definitions and irrevocable replication

Methods

Data Availability

Mark a statement about sample size estimates even if no statistical methods were used

Yes

No statistical method was used to determine sample size.

Were any steps taken to minimize the effects of subjective bias when evaluating evidence from treatment in a randomized procedure? If yes, have these been described?

Yes

We have not used randomization procedures. Samples were analyzed by the scientist performing the experiment.

Is there a separate consent being given to the data being used for the analysis? Were the criteria pre-established?

Yes

We have included a statement that no binding was published in the methods section.

Is the reference list publicly available?

Yes

Yes. A data availability statement is provided at the end of methods section.

Are primary datasets deposited according to the publication guidelines (e.g., Data Deposit Register) and the respective accession numbers provided in the Data Availability Section?

Yes

Yes. A data availability statement is provided at the end of methods section.

Are human clinical and genetic datasets deposited in a public accessible controlled repository in accordance with ethical obligations for the patients and the applicable consent agreement?

Yes

Yes. A data availability statement is provided at the end of methods section.

Is the computational model that was central and integral to a study available without restriction in a machine-readable format? Were the relevant accession numbers or links provided?

Yes

Yes. A data availability statement is provided at the end of methods section.

Could the original data be reproduced by an independent researcher? If so, where?

Yes

Yes. A data availability statement is provided at the end of methods section.

Could any supporting data have been omitted from analysis, except if this was due to ethical or international regulation and privacy restrictions?

Yes

We have only included experiments where the control did not exist.

Could the original data be reproduced by an independent researcher? If so, where?

Yes

Yes. A data availability statement is provided at the end of methods section.

Could the original data be reproduced by an independent researcher? If so, where?

Yes

Yes. A data availability statement is provided at the end of methods section.

Could the original data be reproduced by an independent researcher? If so, where?

Yes

Yes. A data availability statement is provided at the end of methods section.

Could the original data be reproduced by an independent researcher? If so, where?

Yes

Yes. A data availability statement is provided at the end of methods section.

Could the original data be reproduced by an independent researcher? If so, where?

Yes

Yes. A data availability statement is provided at the end of methods section.

Could the original data be reproduced by an independent researcher? If so, where?

Yes

Yes. A data availability statement is provided at the end of methods section.

Could the original data be reproduced by an independent researcher? If so, where?

Yes

Yes. A data availability statement is provided at the end of methods section.

Could the original data be reproduced by an independent researcher? If so, where?

Yes

Yes. A data availability statement is provided at the end of methods section.