THE
EMBO
JOURNAL

Attenuation of PKCδ enhances metabolic activity and promotes expansion of blood progenitors

Tata Nageswara Rao, Manoj K Gupta, Samir Softic, Leo D Wang, Young C Jang, Thomas Thomou, Olivier Bezy, Rohit N Kulkarni, C. Ronald Kahn, and Amy J. Wagers

Review timeline:

Submission date:	6th Aug 2018
Editorial Decision:	17th Aug 2018
Revision received:	31st Aug 2018
Editorial Decision:	7th Sep 2018
Revision received:	8th Sep 2018
Accepted:	12th Sep 2018

Editor: Daniel Klimmeck

Transaction Report:

(Note: An earlier version of this manuscript was assessed by another journal and was then transferred to The EMBO Journal. As the original review of the manuscript was performed outside of The EMBO Journal's transparent review process policy, this Peer Review Process information is not available here. With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 17th Aug 2018

Thank you again for the submission of your manuscript (EMBOJ-2018-100409) to The EMBO Journal. We have carefully assessed your manuscript and the point-by-point response provided to the referee concerns that were raised during re-review at a different journal. In addition, and as mentioned before, we decided to involve an arbitrating expert to evaluate the revised version of your work, with respect to technical robustness, conceptual advance and overall suitability of your work for publication in The EMBO Journal.

As you will see from the report provided below, the arbitrating advisor states the robustness of your work as well as the overall interest and value of your results for the community and s/he thus is supportive of publication at The EMBO Journal.

Based on the positive expert's view together with our own assessment, we conclude that the previous referees' concerns regarding more detailed exploration of the mechanism downstream of PKC-delta in HSPCs and the relevance of this function in additional contexts do not need to be further addressed for publication at The EMBO Journal.

Thus, we decided to proceed with publication of your work at The EMBO Journal pending minor issues related to formatting and data representation as outlined below are conclusively addressed.

Once we have received the revised version, we should then be able to swiftly proceed with formal acceptance and production of the manuscript.

© European Molecular Biology Organization

ARBITRATING ADVISOR'S REPORT:

'The study clearly represents a huge amount of work, with rigorous conditional deletion and serial transplantation studies. I'm not concerned about the increase in HSC proliferation. It's true that increased self-renewal tends to be associated with increased quiescence. However, there are two documented exceptions, e.g. NRas increases the proliferation of a subset of HSCs while increasing overall self-renewal potential. There are also a few examples of genetic modifications that seem to increase proliferation and self-renewal of the overall pool. These examples are particularly interesting. Not much is known yet about HSC metabolism.'

© European Molecular Biology Organization

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Amy J. Wagers
Journal Submitted to: The EMBO journal
Manuscript Number: EMBOJ-2018-100409R

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

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.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- 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-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	A minimum of 4 mice were chosen to consider adequate power to detect a pre-specified effect size. For in vitro experiment, triplicates from each individual mice were tested.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	For experiments involving mice, a minimum of 4-14 mice used where specified. The exact number of mice used in each experiment are indicated in respective figure legeneds.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	In Figure 1 H, Limiting dilution analysis (LDA) Engraftment data shown at 14-weeks post-BMT A recipient mouse was considered positive if donor multilineage engraftment (CD45.2+ blood nucleated cells) exceeded 1% in recipient peripheral blood. Plots show the percentages of recipient mice containing less than 1% CD45.2+ blood nucleated cells. Dotted lines represent the 95% confidence interval of the same (p=0.0005). For the other experiments, no exclusion criteria was applied.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NO
For animal studies, include a statement about randomization even if no randomization was used.	In Figure EV5, WT and PKCd cKO mice (n=12 mice per genotype) were randomized into two groups each containing n=6 mice per genotype and the treatment. For the other experiments, no randomization was performed.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No bias during the group allocation or when assessing results was applied.
4.b. For animal studies, include a statement about blinding even if no blinding was done	No blinding was considered.
5. For every figure, are statistical tests justified as appropriate?	Yes. The stastical measures used for each experiments are indicated in the figure legends and also in the ''Experiments Procedures''.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Statistical analysis Statistical analysis was performed with the use of two-tailed Student's unpaired t-test analysis (when the statistical significance of differences between two groups was assessed) or one-way ANOVAs with subsequent Bonferroni posttest tests (when the statistical significance of differences between more than two groups was assessed), or two-way ANOVAs with subsequent Holm-Sidak's multiple comparison tests with alpha 0.05 as significant (when comparing between groups; for long-term reconstitution assays and for hematopoietic recovery analysis) with Prism software version 6.0 (GraphPad Inc). For the Kaplan-Meier analysis of survival curves, a log-rank nonparametric test (Mantel-Cox test) was performed. Limiting dilution analysis (LDA) was performed with ELDA (http://bioinfo.wehi.edu.au/software/elda/). Significance is denoted with asterisks (*p<0.05, **p<0.01, ***p < 0.001).

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is there an estimate of variation within each group of data?	yes
Is the variance similar between the groups that are being statistically compared?	yes

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	The description about the antibodies used in this study are shown in the Appendix Table 2 in the manuscript.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Noestablished cell lines were used in this study.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Following mouse strains were used in this study:Constitutive PKCδ knockout mice (Leitges et al., 2002); Mx1-Cre+ mice (Kuhn et al., 1995); PKCδ fl/fl/Mx-1Cre- or PKCδ fl/fl/Mx-1Cre+ (this study); B6.SJLPtprca Pep3b/BoyJ (CD45.1) (The Jackson Laboratory). All mice used in this study are between 4-7 months of age, where specified.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All experiments involving mice were performed in accordance with the guidelines set by the Institutional Animal Care and Use Committees (IACUC) of Joslin Diabetes Center and Harvard University.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	All experiments involving mice were performed in accordance with the guidelines set by the Institutional Animal Care and Use Committees (IACUC) of Joslin Diabetes Center and Harvard University.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NO human subjects were used in this study
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NO human subjects were used
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NO human subjects were used
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NO human subjects were used
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NO human subjects were used
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NO human subjects were used
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NO human subjects were used

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Genome wide transcriptomic or proteomic analyses was not performed in this study.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	All the relevant data can be found in the main, expanded view and supplementray figures.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	No human or genomic data sets were included in this study.
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	No computational large data sets were included in this study.

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	All the experiments reported in this study were performed in accordance with the guidelines set by the Institutional Animal Care and Use Committees (IACUC) of Joslin Diabetes Center and Harvard University.