THE EMBO JOURNAL

Notch ligand Dll4 impairs cell recruitment to aortic clusters and limits blood stem cell generation

Cristina Porcheri, Ohad Golan, Fernando J. Calero-Nieto, Roshana Thambyrajah, Cristina Ruiz-Herguido, Xiaonan Wang, Francesca Catto, Yolanda Guillén, Roshani Sinha, Jessica González, Sarah J. Kinston, Samanta A. Mariani, Antonio Maglitto, Chris S. Vink, Elaine Dzierzak, Pierre Charbord, Bertie Göttgens, Lluis Espinosa, David Sprinzak, Anna Bigas

Review timeline:

Submission date:	15th Dec 2019
Editorial Decision:	29th Jan 2020
Revision received:	2nd Feb 2020
Editorial Decision:	4th Feb 2020
Revision received:	5th Feb 2020
Accepted:	6th Feb 2020

Editor: Daniel Klimmeck

Transaction Report:

(Note: Please note that the manuscript was transferred from another journal where it was originally reviewed. Since the original reviews are not subject to EMBO's transparent review process policy, the reports and author response cannot be published. 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 29th Jan 2020

Thank you for sending your manuscript (EMBOJ-2019-104270) to The EMBO Journal for consideration. My apologies again for the unusual delay with the assessment of your manuscript due to protracted expert input. We have carefully evaluated your manuscript and the point-by-point response provided to the referee concerns that were raised during review at a different journal. In addition, we decided to involve an arbitrating advisor to evaluate the revised version of your work, with respect to technical robustness, conceptual advance and overall suitability for publication in The EMBO Journal.

As you will see from the advisor's comments enclosed below, this expert is broadly in favour of the work stating the high interest and value of your results and thus s/he is supportive of publication at The EMBO Journal.

Based on the positive expert's view together with our own assessment, we decided to proceed with publication of your work at The EMBO Journal pending a number of minor points related to manuscript formatting and data presentation as indicated below.

Arbitrating advisor's comments:

I've now read the paper carefully, as well as the reviewer responses and rebuttals. Overall, I am positive about the study and do think it merits publication in EMBO. I agree that the conceptual advance regarding clonality in the clusters is limited, but agree with the authors that presentation of these data were needed in order to take things to the next level. I find the dll4 data intriguing, and while the data presented isn't watertight, I feel it is as good as they can do with existing tools. It is

somewhat unfortunate that the Cre-based labeling efficiency isn't more robust, as this would have allowed them to make stronger conclusions. That said, I think that their interpretations are accurate and likely correct, based both upon the data presented and the correlating mathematical modelling. Finally, I think that the new data presented in response to the reviewer concerns strengthen the paper to the point that it is publishable in its current form.

3rd Editorial Decision 6th Feb 2020

Thank you for submitting the revised version of your manuscript. I have now evaluated your amended manuscript and concluded that the remaining minor concerns have been sufficiently addressed.

Thus, I am pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal.

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: Anna Bigas
Journal Submitted to: The EMBO J
Manuscript Number: EMBOJ-2019-104270R

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 iustified
- 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 χ2 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?	Every experiment was repeated at least 3 independent times.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Sample size was determined by the number of embryos available in each experiment. Transplantatation experiments were repeated until a minimum of 5 animals per group were transplanted. In vitro experiments were repeated until a minimum of 3 embryos in each condition were analysed.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Samples with technical problems such as embryo quality or histological section quality were excluded. Somite number was kept between (32-40 sp) and the rest of embryos were excluded from experiments.
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.	Treated embryos had a similar number of somites to avoid developmental differences. Transplantation experiments were performed in 2-4 month old mice from isogenic strain.
For animal studies, include a statement about randomization even if no randomization was used.	Animals were crossed with inbred colonies and embryos from the same litter and similar number of somites wer used for controls in each experiment. Enough embryos were used to reach a minimum of 3 replicates per experiment.
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.	Quantification of cells and immunofluorescence were performed by at least two blinded people.
4.b. For animal studies, include a statement about blinding even if no blinding was done	No blinding was done for animal studies
5. For every figure, are statistical tests justified as appropriate?	Statistical comparison between two or more groups was performed using an unpaired, Student's T-Test with equal variance. For the mathematical model the Hotelling's T-Squared test multivariate counterpart of the T-Test was used. For comparing distribution of clusters in size categories the non-parametric Mann-Whitney U Test was applied.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	We did not use any test for normal distribution
Is there an estimate of variation within each group of data?	there is no estimate of variation within each group of data

USEFUL LINKS FOR COMPLETING THIS FORM

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

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-tumo

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 the variance similar between the groups that are being statistically compared?	The variance is similar between compared groups.

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).	Catalog number has been provided
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	No 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.	C57BL/6 J wild-type, (Charles River Laboratories), CD41:YFP tg/tg (Zhang et al, 2007),H2B-GFP tg/tg (Hadjantonakis & Papaioannou, 2004) VeCadCreERT (Wang et al, 2010) and R26R-Confetti (Snippert et al, 2010); Gfi1:tomato (Thambyrajah et al, 2016) transgenic lines were all kept under Pathogen free conditions in the PRBB animal facilities. Mainly female mice between 8-16 weeks were used since pregnancy was required and all gender embryos were used.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Procedures for hematopoietic transplantation were approved by the Animal Care Committee of the Parc de Recerca Biomedica de Barcelona (PRBB), Licence number 9309 approved by the Generalitat de Catalunya.
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.	Compliance of ARRIVE guidelines for animal studies.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
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.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
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.	NA
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.	NA

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	Accession codes for datasets have been provided.
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).	Data has been deposited at GEO
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).	NA
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.	The Matlab code for the stochastic simulation is available at https://github.com/OhadGolan/ClusterFormation

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.	NA