THE EMBO JOURNAL

Stable expansion of high-grade serous ovarian cancer organoids requires a low Wnt environment

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Review timeline:

Submission date:	14th Nov 2019
Editorial Decision:	4th Dec 2019
Revision received:	17th Dec 2019
Accepted:	21st Dec 2019

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..)

1st Editorial Decision 4th Dec 2019

Thank you again for the submission of your amended manuscript (EMBOJ-2019-104013) 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 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 the remaining experimental issues currently in question.

In light of the arbitrator's report provided below, together with our own additional reasoning at editorial level we are pleased to inform you that your manuscript has been accepted in principle for publication in The EMBO Journal, pending minor revision of the following aspects, which need to be adjusted in a re-submitted version.

• Complement the study with a more detailed characterization of the Wnt signaling requirements as to the advisor's comments and outlined in your point-by-point response (referee #1, pts.3,4).

• Introduce caveats and relativize where appropriate regarding the claims made on the stemness (referee #2 pt.2) and extended proof of translational value of the study (referee #3).

• We are piloting Structured Methods a new format for the Materials and Methods of articles published at EMBO Press. Adhering to this format is optional for research articles. However, considering the strong methodological aspect of your study, we would strongly encourage you to use it. Specifically, the Material and Methods section should include a Reagents and Tools Table (listing key reagents, experimental models, software and relevant equipment and including their sources and relevant identifiers) followed by a Methods and Protocols section in which we encourage the authors to describe their methods using a step-by-step protocol format with bullet points. More information on how to adhere to this format as well as downloadable templates (.doc or .xls) for the Reagents and Tools Table can be found in the author guidelines of our sister journal Molecular Systems Biology http://msb.embopress.org/authorguide#materialsandmethods. An example of a paper with Structured Methods can be found here: http://msb.embopress.org/content/14/7/e8071. We encourage you to be even more explicit in adding details on the experimental procedures, as this should be

valuable in ensuring reproducible application if the approach.

We expect the above mentioned additional experiments to be realistic for a minor revision.

Based on the overall positive expert's view together with our own assessment, we decided to proceed with publication of your work at The EMBO Journal pending the above points related to the advisor's input could be conclusively addressed in a time frame of two weeks.

Arbitrating advisor's comments:

>> Wnt inhibition: (reviewer 1, points 3 and 4): I think it would be important to extend further the observation that WNT signaling is detrimental to Ovarian Cancer Organoids as this goes against most of the published organoid protocols, where WNTs are essential. Treatment with CHIR is a straight forward experiments. Genetic downregulation of Kremen and Dkk3 is also feasible although more cumbersome. However, other genetic and pharmacological experiments could also be used to reinforce this part.

>> Extent of stemness characterisation (reviewer 2 point 2): I do not see this as essential part. Markers of ovarian stem cells are not well known anyway.

>> Additional drug treatment data (reviewer 3). I agree with this referee that the study of single agent treatment is too simplistic and that demonstration that this organoid method captures heterogenous responses to several classes of commonly used types of chemotherapy would increase the relevance of the work. However, comparison of organoid responses to patient responses is probably out of the scope of the present work as it may require analyses with a large cohort of samples to reach significant conclusions.

1st Revision - authors' response 17th Dec 2019

• Complement the study with a more detailed characterization of the Wnt signaling requirements as to the advisor's comments and outlined in your point-by-point response (referee #1, pts.3,4).

As we have indicated in our previous point-by-point response we did test an alternative mode of Wnt signalling activation by addition of GSK3-β *inhibitor CHIR99201 during the optimization phase of determining conditions for patient-derived HGSOC organoid cultures and found it has a strong inhibitory influence on organoid formation and growth. We have included this data now in the manuscript (Fig EV1B, Results: lines 123-125). We also address this point in the discussion section, as we agree it provides an important independent confirmation that canoncial Wnt signalling activation is detrimental for HGSOC organoids without relying on the usage of conditional Wnt3a medium.*

Lines 372: "Importantly, activation of Wnt pathway through the action of the GSK3-β inhibitor CHIR also inhibits organoid growth, which independently confirms the functional relationship between low Wnt signal and maintenance of HGSOC growth potential in vitro."

Table EV3 also contains a detailed list of all different signalling conditions which were tested during the establishment of the cancer organoid cultures.

In addition, we made an effort in providing a clear context in the manuscript for the interpretation of our findings of KREMEN2 and DKK3 upregulation that we have observed in HGSOC and KD FT organoid culture as well as in cancer tissue samples. We recognize that on its own this data is insufficient to claim the existence

of a causal relationship. Accordingly, we worded this carefully in the manuscript and point to different mechanisms that are potentially involved in the process of Wnt inhibition.

Line 339: "The expression of Wnt inhibitors and the reduced Wnt signaling induced by tumor suppressor KD are in agreement with our hypothesis that Wnt pathway suppression is required for HGSOC growth, which can be achieved by different molecular mechanisms, e.g. by cell autonomous means or changes in the niche environment."

• Introduce caveats and relativize where appropriate regarding the claims made on the stemness (referee #2 pt.2) and extended proof of the translational value of the study (referee #3).

We have done so accordingly by introduced a clarifying sentence in the discussion part and providing suitable references:

Line 422: "Also, while we show that surface expression of CD133 correlates with in vitro growth capacity of HGSOC organoids, the exact functional relationship between stemness regulation in organoid culture and other previously described ovarian cancer stem cell markers CD117, ALDH1 and CD24 are yet to be tested."

We have also now clearly formulated that our study only provides a starting model for in vitro drug testing and that further more comprehensive analysis is needed to assess its translational potential.

Line 433: "However, to define to which extent organoids are predictive of in vivo patient responses, more comprehensive in vitro drug testing studies in correlation with the long-term clinical outcome are needed. In order to identify how stem cells respond to cancer therapy, which is of critical importance for the development of new treatment approaches, paired organoid cultures could be generated from primary disease and after recurrence in the same patients."

We believe that long-term culture of patient-derived organoids provides a valuable tool for advancing therapeutic options in cancer research, but as pointed out by an advisory reviewer, this issue was out of the scope of the main question we investigated which is regulation of stemness in HGSOC organoids and changes in FT epithelium homeostasis during the transformation process.

2nd Editorial Decision 21st Dec 2019

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

Mirjana Kessler, Thomas F. Meyer

EMBO Journal

Manuscript Number: EMBOJ-2019-104013

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?	The number of ovarianc cancer samples was chosen based on availability of samples and resources for analysis in an exploratory setting. No minimal effect sizes were pre-defined.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	According to predefined inclusion criteria, only female patients older than 18ys were included after informed consent.
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.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
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.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Statistical tests are stated where appropriate under each figure and in Methods and Protocols under the section 'Statistical analysis' and for gene expression and sequencing data under the sections 'Microarrays' and 'Capture of the targeted disease-related genome and Next-Generation Sequencing', respectively.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Due to the small sample size used in statistical tests, no statistical methods were used to test the assumption of the tests.

USEFUL LINKS FOR COMPLETING THIS FORM

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Is there an estimate of variation within each group of data?	Standard error of the mean (SEM) is provided to indicate variation of data in figures as described in figure legends.
Is the variance similar between the groups that are being statistically compared?	A variant of t-test for unequal variances was used throughout.

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).	For all antibodies a catalogue number and/or clone number is provided in the Reagents and Tools table.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	The source of the cell lines used in this study is given in the Reagents and Tools table and all lines were subjected to mycoplasma contamination tests prior to use 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.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
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.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Approval for the preparation and experimental usage of the primary material was given by the Ethics Commission of the Charité, Berlin (EA1/002/07).
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.	Informed consent was obtained from every patient for collection and processing of samples in agreement with the Declaration of Helsinki.
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	All microarray data have been deposited in the Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo/) (GEO; www.ncbi.nlm.nih.gov/geo/) of the National Center for Biotechnology Information and can be accessed with the GEO accession numbers GSE125883 (Reviewer token: yhqtyomkznsjdkd; dual-color microarrays) and GSE124766 (Reviewer Token: ubwhqiusjzwpxez; single-color microarrays). Sequence data has been deposited at the European Genome-phenome Archive (EGA) under accession number EGAS00001003821 (https://www.ebi.ac.uk/ega/studies/EGAS00001003821). All code used for generating analyses in this publication is available from https://github.com/MPIIB-Department-TFMeyer/Hoffmann_et_al_Ovarian_Cancer_Organoids.
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).	NA
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).	Sequence data has been deposited at the European Genome-phenome Archive (EGA) under accession number EGAS00001003821 (https://www.ebi.ac.uk/ega/studies/EGAS00001003821).
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.	NA

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