EMBO Molecular Medicine

Contraceptive progestins with androgenic properties stimulate breast epithelial cell proliferation

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1st Editorial Decision 24th Mar 2021

Thank you for submitting your manuscript to EMBO Molecular Medicine. I have now carefully read your manuscript and the response to the reviewers, and discussed them with the other members of our editorial team. In addition, I have also consulted a member of our Editorial Advisory Board. I am pleased to inform you that we find your manuscript suitable for publication in EMBO Molecular Medicine.

Please read below for important editorial formatting and consult our author's guidelines for proper formatting of your revised article for EMBO Molecular Medicine.

1st Authors' Response to Reviewers 5th Apr 2021

The authors have made all requested editorial changes.

1st Revision - Editorial Decision 9th Apr 2021

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. I am pleased to inform you that we will be able to accept your manuscript pending the following editorial level amendments:

2nd Authors' Response to Reviewers 15th Apr 2021

The authors have made all requested editorial changes.

Accepted 23rd Apr 2021

23rd Apr 2021

I am pleased to inform you that your manuscript is accepted for publication and is now being sent to our publisher to be included in the next available issue of EMBO Molecular Medicine.

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: Cathrin Brisken, MD, PhD
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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?	Sample size was estimated based on previous experience with the experimental approaches (Sflomos, G. et al., Cancer Cell, 2016).
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	No statistical test was used to estimate the sample size. Based on our experience, for growth curves, at least 2 mice with more than two mammary glands injected per mouse (therefore >4 mammary glands).
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No inclusion/exclusion criteria were applied to our cohorts.
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.	Before initiation of treatment, we ensured that control and treatment cohorts have similar radiance values. In case of relevant discrepancies, mice were randomized to reach equal radiance in both cohorts.
For animal studies, include a statement about randomization even if no randomization was used.	Mice were randomized according to the radiance of mammary glands.
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.	N/A
4.b. For animal studies, include a statement about blinding even if no blinding was done	Investigators were not blinded to group allocation.
5. For every figure, are statistical tests justified as appropriate?	For growth curves analysis, statistical tests were run by the lab's bioinformatician/biostatistician, Dr. Giovanna Ambrosini as described on the figure legends. For comparison between two groups, typically Student t-tests (paired or unpaired) were used depending if the data sets follows a random distribution. Wilcoxon sum ranked test (or Mann Whitney test) has been used when comparing two independent samples when the outcome is not normally distributed and the samples are small.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	For small sample size (i.e. <6), Shapiro-Whilk normality tests were used to determine normality. We prefer to use non-parametric tests for small sample size.

USEFUL LINKS FOR COMPLETING THIS FORM

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http://oba.od.nih.gov/biosecurity/biosecurity_documents.html	Biosecurity Documents from NIH
http://www.selectagents.gov/	List of Select Agents

Is there an estimate of variation within each group of data?	Data represent the mean ± SD or SEM. This is included in the figure legends.
Is the variance similar between the groups that are being statistically compared?	F-tests were checked on graphpad prism software. Depending on the datasets, variance was similar among groups and t-tests with or without equal variances were used accordingly. Levene's test has been sometimes used to test for equalitarianism of variances among 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).	Primary Antibodies: Ki67 (rabbit α-Ki67, clone Sp6, Spring Amsbio, dilution 1:200), ER (rabbit α-ER, clone Sp1, Zytomed RTU), PR (rabbit α-PR, clone 1E2, Ventana RTU), AR (rabbit α-AR, clone SP107, Thermo Fisher, diluted 1:200), Cytokeratin7 (rabbit α-CK7, clone Sp52, Abcam, diluted 1:500), Cytokeratin 19 (mouse, KS19.2(Z105.6), American Research Products Inc. (ARP), 1:50), and E-Cadherin (mouse α-ECAD, clone G-10, sc-8426, diluted 1:100)
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 for 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.	Information privded at page 28. NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice were purchased from Charles River and The Jackson Laboratory. All mice were maintained and handled according to Swiss guidelines for animal safety with a 12-h-light-12-h-dark cycle, controlled temperature and food and water ad libitum.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Information privded at page 28. Experiments were performed in accordance with protocol VD1865.5 approved by Service de la Consommation et des Affaires Vétérinaires, Canton de Vaud, Switzerland.
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.	We confirm compliance.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Information privded at page 29. The Commission cantonale d'éthique de la recherche sur l'être humain (183/10) approved 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.	Information privded at page 29. Informed consent was obtained from all subjects.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
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.	N/A
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.	N/A

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	We provide this information in a specific paragraph names "Data Availability" in the Materials and Methods Section, page 33. "Gene Expression Omnibus (GEO) accession number for the transcriptomics data reported in this study is GSE155274 ; GEO SubSeries accession numbers referring to human microstructures and mouse organoids experiments are GSE155272 and GSE155273, respectively."
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).	N/A
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).	N/A
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.	N/A

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.	N/A