EMBO Molecular Medicine

CDK4/6 inhibition mitigates stem cell damage in a novel model for taxane-induced alopecia

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Transaction Report:

(Note: 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 19 July 2019

Thank you for the submission of your manuscript to EMBO Molecular Medicine. I apologise for the delay in reaching a decision. Although I was hoping to obtain a third evaluation, I am now proceeding based on the two consistent evaluations obtained so far as further delays cannot be justified. I will forward Reviewer 2's delayed report, if and as soon as we are able to obtain it. When (within reason) this report does arrive and if it raises additional important issues that have to be addressed to support this study, these would also need to be taken into consideration in your revision. Please note that I would not ask you to consider further-reaching requests with respect to the current evaluations.

As you will see from the comments below, the referees are enthusiastic about the study and have suggestions and recommendations to further improve clarity as well as increase the potential clinical implications with a thorough discussion as suggested, which is particularly important for our scope.

We would therefore welcome the submission of a revised version within three months for further consideration and would like to encourage you to address all the criticisms raised as suggested to improve conclusiveness and clarity. Please note that EMBO Molecular Medicine strongly supports a single round of revision and that, as acceptance or rejection of the manuscript may depend on another round of review, your responses should be as complete as possible.

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

Please also contact us as soon as possible if similar work is published elsewhere. If other work is published we may not be able to extend the revision period beyond three months.

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

I look forward to receiving your revised manuscript.

***** Reviewer's comments *****

Referee #3 (Comments on Novelty/Model System for Author):

These are human explants of hair follicles, which are suited for this study

Referee #3 (Remarks for Author):

In this manuscript the authors investigate chemotherapy-induced alopecia by taxanes. This is an interesting topic. The authors convincingly show that taxanes induce a mitotic arrest in hair follicles but overall proliferation is not affected. Eventually, this leads to apoptosis, which is not surprising. Treatment of hair follicles with the CDK4/6 inhibitor palbociclib induced a G1 arrest and protected hair follicles from entering mitosis and apoptosis. The authors tried different regimens with similar results, which underscores that CDK4/6 inhibitor treatment could work in preventing of chemotherapy-induced alopecia maybe even in human patients down the road.

Overall, I enjoyed reading this manuscript since it is present well and contains valuable data. It is an important contribution in the understanding of alopecia and the mechanism of CDK4/6 inhibitors, which are FDA approved to be used in the clinic.

There are a number of issues, which need to be addressed:

1. The authors should try to use less abbreviations...it really makes the reading more difficult than needed and does not serve any real purpose. For example, HF, ORS, CIA (especially this one made me smile!), etc.
2. My feeling is that Figure 1C and 1D should be combined in one graph. It would make reading the data easier.
3. The data for Figure 2D should be quantified as shown in Figure 2A. From the description, the numbers are less impressive than for Paclitaxel but it is still important to have these numbers.
4. For each Figure is should be mentioned in the Figure legends, how many cells were counted. This is essential for the understanding of the data.
5. In Figure 3B, the data is plotted inversely which is confusing. The Paclitaxel treated samples should have a lower count for RNA synthesis.
6. The pictures (data) for Figure 4E needs to be shown. This can be done as supplemental data/figure.
7. The last 3 figures are almost the same but the experiments are different. The authors should try to describe these experiments in a way that keeps the attention of the reader high. Otherwise, the reader gets lost.

Referee #4 (Comments on Novelty/Model System for Author):

Chemotherapy has many devastating side effects on proliferative healthy organs including digestive tract, bone marrow, kidney and skin. Chemotherapy-induced hair loss is one of these highly distressing adverse side effects. The research community is confident that very soon we will significantly increase efficacy of the cancer treatment and in parallel we should address a challenge how to reduce its undesired effects.
In this manuscript, Purba et al. examine the implication of a group of chemotherapeutic agents, Taxanes, in detrimental effects on human scalp hair follicles using ex vivo organ culture model. In addition, for the first time the authors experimentally test G1 cell cycle arrest therapy as a preventive treatment in taxane-induced hair follicle damage.
Presented proof-of-principal experiments are very well organized and very informative together with established methods of detection of proliferation, apoptosis and protein synthesis. Developed ex vivo assay could be very useful for studying and experimentally manipulating toxicology in healthy human hair follicles.
The authors show that mitosis-targeting paclitaxel and docetaxel affect highly proliferative hair forming matrix keratinocytes and Keratin 15 positive epithelial stem/progenitor cell niches located in upper outer root sheath portion of the hair follicle. The authors note, that normally slow cycling stem cells become more proliferative in culture. The fact should be discussed in relevance to

permanent chemotherapy induced hair loss when patient undergoes multiple treatment sessions with possible damage of hair follicle stem cells occurs during activation of a new growth cycle followed by initial chemotherapy-induced dystrophic catagen (Paus et al., Lancet Oncol 2013).
Then authors tested the hypothesis that pharmacologically induced cell cycle arrest protects against taxane-induced human hair follicle damage. The G1 arresting CDK4/6 inhibitor, palbocilib, protected paclitaxel-induced cytotoxicity in proliferative epithelial progenitor compartments. It's very exciting model to test further.

1st Revision - authors' response 5 August 2019

******* Reviewer's comments ***** Referee #3.**

Thank you so much for your encouraging comments and for providing very helpful constructive suggestions to improve our manuscript.

1. The authors should try to use less abbreviations...it really makes the reading more difficult than needed and does not serve any real purpose. For example, HF, ORS, CIA (especially this one made me smile!), etc.
Done as requested: the abbreviations mentioned (i.e. CIA, ORS, HF) have been removed from the main body of text to enhance readability.

2. My feeling is that Figure 1C and 1D should be combined in one graph. It would make reading the data easier.
Thank you for this suggestion. We have altered the labeling of the graphs to more clearly distinguish data obtained from different experiments to make the data easier to read. Please see revised graphs in Figure 1C and 1D.
As the control data from graphs 1C and 1D are from distinct organ culture experiments (i.e. using hair follicles from unique donors), it would not be ideal to combine this data within the graph as one single control group, as the data would not be directly comparable. However, we'd be happy to generate a combined figure, if the editors advise to do so.

3. The data for Figure 2D should be quantified as shown in Figure 2A. From the description, the numbers are less impressive than for Paclitaxel but it is still important to have these numbers.
Thanks – agreed. As requested, we have performed this quantitative analysis, and are now presenting the data in a new figure (Figure 2B).

4. For each Figure is should be mentioned in the Figure legends, how many cells were counted. This is essential for the understanding of the data.
Within each graph, each value plotted represents the mean number of cells counted for a given parameter (e.g. Ki-67 labeling) per hair follicle analyzed (within a defined reference region, consistently applied to control and treated conditions). This has now been further clarified in the main text figure legends (pages 33-38) and in the methods (page 22).
Including this information (i.e. number of cells counted) again within the legend would be superfluous, unless we have misunderstood the request of the reviewer, in which case we would be happy to accommodate this request upon further clarification.

5. In Figure 3B, the data is plotted inversely which is confusing. The Paclitaxel treated samples should have a lower count for RNA synthesis.
Thanks for pointing this out. We have reanalyzed the data so that the effects of paclitaxel treatment on RNA synthesis are presented more clearly. Please see our new graph in revised Figure 3B.

6. The pictures (data) for Figure 4E needs to be shown. This can be done as supplemental data/figure.
As requested, please see Figure 4E for images corresponding to the quantitative data that is now presented in Figure 4F.

7. The last 3 figures are almost the same but the experiments are different. The authors should try to describe these experiments in a way that keeps the attention of the reader high. Otherwise, the reader gets lost.

Thanks for this suggestion for improvement. We have edited the results section corresponding to the final 3 figures to more clearly describe and distinguish the conditions between the experiments to improve reading clarity. Please see main text, pages 11-15.

Referee #4 (Comments on Novelty/Model System for Author):

Thanks a lot for the very constructive feedback.

The authors show that mitosis-targeting paclitaxel and docetaxel affect highly proliferative hair forming matrix keratinocytes and Keratin 15 positive epithelial stem/progenitor cell niches located in upper outer root sheath portion of the hair follicle. The authors note, that normally slow cycling stem cells become more proliferative in culture. The fact should be discussed in relevance to permanent chemotherapy induced hair loss when patient undergoes multiple treatment sessions with possible damage of hair follicle stem cells occurs during activation of a new growth cycle followed by initial chemotherapy-induced dystrophic catagen (Paus et al., Lancet Oncol 2013).
Thank you for this excellent suggestion. We have now elaborated on this important discussion point within our revised discussion section. Here, we also describe that our *ex vivo* test system is limited in addressing the hair follicle stem cell response to fractionated, repetitive chemotherapy cycles and that mouse models with xenotransplanted human scalp hair follicles that permit long-term observations *in vivo* are required. Please see Discussion, pages 19-20.

Then authors tested the hypothesis that pharmacologically induced cell cycle arrest protects against taxane-induced human hair follicle damage. The G1 arresting CDK4/6 inhibitor, palbocilib, protected paclitaxel-induced cytotoxicity in proliferative epithelial progenitor compartments. It's very exciting model to test further
Thank you again for your positive feedback

2nd Editorial Decision 7 August 2019

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

2nd Revision - authors' response 13 August 2019

The authors performed all minor editorial changes.

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: Talveen Purba
Journal Submitted to: EMBO Molecular Medicine
Manuscript Number: EMM-2019-11031

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.

USEFUL LINKS FOR COMPLETING THIS FORM

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

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

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?	No power calculation was performed.
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?	NA
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.	Hair follicles were randomly allocated to a given treatment group.
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.	Repeat analysis of data was performed independently by distinct investigators to verify treatment effects on a given parameter.
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 testing employed the unpaired t test for normally distributed data (or Welch's test for data with unequal variances), or the Mann Whitney U test.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Normality testing was performed using D'Agostino-Pearson omnibus test.

Is there an estimate of variation within each group of data?	Standard error was used as appropriate for graphs comparing treatment groups to control
Is the variance similar between the groups that are being statistically compared?	Welch's test [was employed] for data with unequal variances

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).	See - Purba TS et al. A primer for studying cell cycle dynamics of the human hair follicle. Exp. Dermatol. 2016;25(9):663-8 and Purba TS et al. Divergent proliferation patterns of distinct human hair follicle epithelial progenitor niches in situ and their differential responsiveness to prostaglandin D2. Sci. Rep. 2017;7(1):15197
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* 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.	Experiments within this study were performed under ethical approval granted by the University of Manchester (REC reference 19/NW/0082) or University of Muenster (n.2015 -602-f-S).
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.	Samples were obtained after informed consent and ethics committee approvals and treated according to the Helsinki Ethical Guidelines for medical research involving human subjects, under human tissue act guidelines.
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	NA
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).	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.	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