EMBO reports

MSCs Rescue Impaired Wound Healing in a Murine LAD1 Model by Adaptive Responses to Low TGF-β1 Levels

Dongsheng Jiang, Karmveer Singh, Jana Muschhammer, Susanne Schatz, Anca Sindrilaru, Evgenia Makrantonaki, Yu Qi, Meinhard Wlaschek, Karin Scharffetter-Kochanek

Review timeline:

Submission date:	21 August 2019
Editorial Decision:	22 November 2019
Revision received:	9 December 2019
Accepted:	31 January 2020

Editor: Martina Rembold

Transaction Report: This manuscript was transferred to *EMBO reports* following peer review at *EMBO Molecular Medicine.*

(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 22 November 2019

Thank you for the submission of your research manuscript to our journal. The study has now been reviewed by former referee 1 who also had access to the referee reports from the other journal and your point-by-point response. As you can see from the comments below, the referee finds the revised study now suitable for publication in EMBO reports.

We therefore invite you to revise your study for publication in EMBO reports and to address the following editorial points that we need before we can proceed with the official acceptance of your study.

1) Please submit your manuscript as a .docx formatted version that contains only the text (including legends for main figures, EV figures and tables).

2) Please shorten the title to 100 characters including spaces and the abstract to 150 words.

3) We need individual production quality figure files as .eps, .tif, .jpg (one file per figure).
Please also see our Figure Preparation Guidelines (figure preparation pdf) from our Author Guidelines pages
https://www.embopress.org/page/journal/14693178/authorguide for more info on how to prepare your figures.

4) Supplementary Information: you currently have 6 Supplementary figures. You have two options:
- You could combine them to 5 figures and submit them as Expanded View (EV) Figures. These are collapsible/expandable online. Table S1 could also be displayed in Expanded View. If you choose this option, we need the EV Figures as individual production quality files and the EV Figure legends should be included in the main text after the legends of regular figures. The nomenclature is "Figure EVx", "Table EVx".
- Alternatively, you can combine all Supplementary figures/tables and their legends into a single pdf called "Appendix". The nomenclature for these is "Appendix Figure Sx", "Appendix Table Sx".

The Appendix needs a title page with a table of content including page numbers.

See detailed instructions regarding expanded view here:
<https://www.embopress.org/page/journal/14693178/authorguide#expandedview>

5) Figure legends and statistics:
All figure legends must contain the following information:
- Graphs must include a description of the bars and the error bars (SEM, SD).
- The statistical test used to generate the error bars and P-values must be stated.
- The number of independent biological replicates underlying each data point must be given.
IMPORTANT: please note that error bars and statistical comparison may only be applied if the data are based on at least 3 independent biological replicates. If your data does not meet these criteria, either provide more samples or show the data as scatter blots.
- All microscopy images must contain a scale bar that is defined in the legend.

6) Please complete and upload the author checklist, which you can download from our author guidelines (<https://www.embopress.org/page/journal/14693178/authorguide>). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

7) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (<https://orcid.org/>). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines (<https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>)

8) EMBO reports papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is 550x200-400 pixels large (width x height). You can either show a model or key data in the synopsis image. Please note that the size is rather small and that text needs to be readable at the final size. Please send us this information along with the revised manuscript.

9) We would also encourage you to include the source data for figure panels that show essential data. Numerical data should be provided as individual .xls or .csv files (including a tab describing the data). For blots or microscopy, uncropped images should be submitted (using a zip archive if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available
<https://www.embopress.org/page/journal/14693178/authorguide#sourcedata>.

10) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at
<https://www.embopress.org/page/journal/14693178/authorguide#referencesformat>.

11) As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics

Illustrator in designing a cover.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

****

REFEREE REPORTS

Referee #1:

The study by Jiang and coworkers has previously been submitted to EMBO Molecular Medicine and was reviewed by this reviewer. The authors addressed all technical concerns either with new experiments and data or by down-toning interpretations that were not well substantiated. They have included essential references in their discussion and experimental considerations.

One major initial concern was the lack of conceptual novelty of the first submission considering the substantial work already published on the roles of TGF and miR-21 in a wound healing context. However, the authors now better support and discuss that MSCs delivered to a wound bed can adapt their levels of TGF production to the local environment. Their data support that miR-21 and Smad7 are involved in this rheostat functions and that this function is differently regulated in MSCs compared with dermal fibroblasts. The manuscript has been restructured and writing has been improved which clarified most if not all initial uncertainties.

In summary, the initial submission the work has been very much improved and is consistent with the standards requested by EMBO Reports. This reviewer has no further concerns.

1st Revision - authors' response 9 December 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: Karin Scharffetter-Kochanek
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2019-49115V1

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?	Not specified.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Sample size for in vivo studies was estimated with experiences established in previously published peer-reviewed studies. For example, for wound size measurement, about 20 wounds per treatment group was assessed.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	In general, no sample or animal was excluded from the analysis. Techinical controls were used to dertermine whether a specific experiment was technicall sound. Samples showing obvious technical artefacts were not included into the analysis.
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.	Mice within a same strain (wildtype or CD18-deficient) were randomly selected and allocated to either control or experimental groups. Mice between groups were gender- and age-matched.
For animal studies, include a statement about randomization even if no randomization was used.	The information is indicated in the Materials and Methods section.
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.	For wound size analysis, the researchers who performed imaging analysis were blinded to the mice grouping information.
4.b. For animal studies, include a statement about blinding even if no blinding was done	The statement is included in the Materials and Methods section.
5. For every figure, are statistical tests justified as appropriate?	As stated in the corresponding figure legends, two-tailed unpaired t-tests with Welch's correction, or nonparametric Mann-Whitney tests were used to determine statistical significance between two groups; and one-way ANOVA with Tukey's multiple comparison tests were used to determine the statistical significance in multiple groups.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	The two-tailed unpaired t-tests with Welch's correction, or nonparametric Mann-Whitney tests were performed.

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. As stated in the corresponding figure legends, data were expressed as mean +/- SEM or mean +/- SD. The error bars indicate variation within each group of data.
Is the variance similar between the groups that are being statistically compared?	If variances between groups were different, appropriate corrections were used, which are indicated in the figure legends.

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 clone numbers and catalog numbers for all the antibodies used in Western blot and immunofluorescence staining were specified in the Materials and Methods section.
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 primary human adipose tissue-derived mesenchymal stem cells and human dermal fibroblasts used in this study were tested for mycoplasma-free status.

* 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.	This information is indicated in the Materials and Methods section.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All experiments were carried out in compliance with the German Law for Welfare of Laboratory Animals. The animal experiments were approved by the government of Baden-Württemberg with project numbers 117 and 1396.
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.	Animal experiments were carried out in compliance with the ARRIVE reporting guidelines. This information is indicated in the Materials and Methods section.

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	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