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Special issue article <Negative results>

CCAAT/enhancer binding protein delta (C/EBPδ) deficiency does not affect bleomycin-induced pulmonary fibrosis

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1st editorial decision
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Ref.: Ms. No. JCTRes-D-17-00019
CCAAT/enhancer binding protein delta (C/EBPδ) deficiency does not affect bleomycin-induced pulmonary fibrosis.
Journal of Clinical and Translational Research

Dear authors,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you resubmit your work.

Your revision is due by Dec 24, 2017.

To submit a revision, go to http://jctres.edmgr.com/ and log in as an Author. You will see a menu item called Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

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Reviewers' comments:

Reviewer #1: In this paper, the Authors demonstrate that C/EBPd deficient mice do not display increased or decreased features of lung fibrosis following bleomycin exposure, supporting the hypothesis that this pathway might not be a relevant target in IPF.

Major concerns:
- The Authors should perform IHC or IF for C/EBPd in order to identify expressing cells in the lung.

Minor concern:
- Paragraph 1, line 13 (introduction); in fact both pirfenidone and nintedanib demonstrated a clear effect on lung function decline, I would remove the world "seem". Similarly, dugs had a mild but signifiant effet on Saint George Respiratory Questionnaire (QoL).

Reviewer #2: The present work aims to question the importance of CCAAT/enhancer binding protein delta (C/EBPδ) during pulmonary fibrosis. First, the authors showed that C/EBPδ expression is reduced in the lung of patients with IPF compared with control patients. Then they tested the importance of C/EBPδ expression in bleomycin-induced lung fibrosis using C/EBPδ deficient mice before focusing on macrophage recruitment and polarization following bleomycin challenge. The manuscript is clearly and elegantly written.

Major comment:
Overall, the methods and experimental setups (number of biological and technical replicates should be presented in the figure legends) are incomplete or lacking and have to be better defined for each experiment. Better description of the human samples used in this study needs to be included in the manuscript (smocking history, lung function parameters…).
A deeper investigation should be performed for the bleomycin model. Please consider adding saline control for both C/EBPδ null mice and wild-type. Analysis of the BALF (TGF-beta1 level, differential count, inflammation assessment) is missing in the study.
The study of macrophage polarization based on qPCR in whole lung extracts should be confirmed. The authors should extract lung macrophages and assess both macrophage recruitment and polarization using flow cytometry.

Minor points:
Interestingly, authors discuss a potential compensation of C/EBPδ by other member(s) of the C/EBP factor family. Please assess mRNA levels of the other C/EBP members in IPF as well as in the C/EBPδ deficient mice (both at baseline and following bleomycin and compared with wild-type littermates).
qPCR data are normalized using UBC (human) or Tbp (mouse). Please provide data showing the constancy of expression of these genes among the groups.
Statistical analysis need to be clearly stated in the figure legends. Please implement p-value for each comparison.

********Authors' rebuttal********

Dear editor,

Please find enclosed a resubmission of our manuscript (JCTRes-D-17-00019) entitled "CCAAT/enhancer binding protein delta (C/EBPδ) deficiency does not affect bleomycin-induced pulmonary fibrosis." We have appreciated the reviewers' comments and addressed the concerns raised. We feel our manuscript substantially improved as a consequence thereof. Hopefully you concur and feel this version of the manuscript is suitable for publication in JCTRes. Below you will find a

point-by-point response to each of the reviewer comments. All changes are indicated in red in the revised manuscript (track changes).

Kind regards (on behalf of all authors),
JWD

Reviewer #1:
"Major concerns:
- The Authors should perform IHC or IF for C/EBPd in order to identify expressing cells in the lung." – We have performed IHC for C/EBPδ and the results are added to the manuscript (Figure 1b-c).

Minor concern:
- Paragraph 1, line 13 (introduction); in fact both pirfenidone and nintedanib demonstrated a clear effect on lung function decline, I would remove the world "seem". Similarly, dugs had a mild but significant effect on Saint George Respiratory Questionnaire (QoL). – We agree with the reviewer and have modified the sentence in the manuscript to: "pirfenidone and nintedanib, which both significantly reduce the decline of lung function in patients with mild to moderate IPF were introduced into the clinic [4,5]. However, both drugs have serious side effects and do not stop nor reverse the disease."

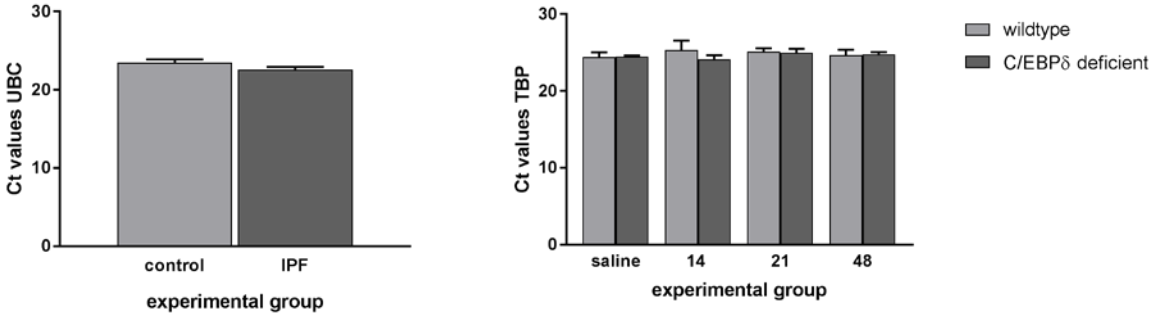
Reviewer #2:
Major comment:
- Overall, the methods and experimental setups (number of biological and technical replicates should be presented in the figure legends) are incomplete or lacking and have to be better defined for each experiment. Better description of the human samples used in this study needs to be included in the manuscript (smocking history, lung function parameters…). – The number of replicates for each experiment have been added to the figure legends and a table with patient demographics, including FVC and smoking history, has been added to the manuscript (table 1).

- A deeper investigation should be performed for the bleomycin model. Please consider adding saline control for both C/EBPδ null mice and wild-type. – Data obtained from saline treated mice have been presented in the manuscript.

- The study of macrophage polarization based on qPCR in whole lung extracts should be confirmed. The authors should extract lung macrophages and assess both macrophage recruitment and polarization using flow cytometry. – Although confirmation of qPCR data by protein analysis may indeed be important in general, we feel that protein data do not improve the quality of the manuscript as it will not add to the message of the paper, i.e. no effect of C/EBPδ on pulmonary fibrosis during bleomycin-induced pulmonary fibrosis. Including protein data would involve additional animal experiments which we feel are not appropriate from an ethical perspective (again as it will not change the message of the manuscript although minor differences on macrophage polarization and recruitment could be observed).

Minor points:
- Interestingly, authors discuss a potential compensation of C/EBPδ by other member(s) of the C/EBP factor family. Please assess mRNA levels of the other C/EBP members in IPF as well as in the C/EBPδ deficient mice (both at baseline and following bleomycin and compared with wild-type littermates). – Based on in silico analysis of publically available GEOdatasets, we identified C/EBPβ to be the most likely candidate for the compensatory loss of C/EBPδ. Subsequent qPCR analysis of C/EBPβ expression revealed that C/EBPβ is indeed abundantly expressed in both wildtype and C/EBPδ deficient mice (Figure 5).

- *qPCR data are normalized using UBC (human) or Tbp (mouse). Please provide data showing the constancy of expression of these genes among the groups.* – Ct values for *ubc* were consistent between control and IPF patients whereas Ct values for *tbp* were consistent between experimental groups of the mouse experiment (see figure below).



- *Statistical analysis need to be clearly stated in the figure legends. Please implement p-value for each comparison.* – A description of the statistical analysis used is presented in the material and methods section of the manuscript. We apologies for not describing the depicted asterisks indicating significant differences between experimental groups and have corrected this omission in the revised manuscript.

2nd editorial decision
Date: 1 Feb, 2018

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CCAAT/enhancer binding protein delta (C/EBPδ) deficiency does not affect bleomycin-induced pulmonary fibrosis.
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Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.
You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.
Thank you for submitting your work to JCTR.
Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers:

Reviewer #1: The Authors have properly addressed my comments and the manuscript has improved accordingly.
Reviewer #2: The authors have addressed many of the previous comments. I understand they did not perform deeper investigation on macrophage polarization as initially suggested. Nevertheless, the paper has been improved.
Please correct the name of table 2.

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