



17.11.2015

Reykjavik, Iceland.

To

Dr. Pankaj Goyal,

Editor PeerJ,

Dear Editor,

We would like to thank all the reviewers for their positive comments on our manuscript. We have attached a response to the comments from reviewer 1. Although, reviewer 1 suggests representing the Y-axis for q-RT PCR data on a \log_2 scale, we would like to draw your attention to the fact that the data as presented now on Y-axis is not log transformed. Hence we cannot label Y-axis as \log_2 relative expression. We have explained in details the interpretation of Y-axis in figure legends for the readers to have more clarity. The method we used for representing q-RT PCR data is widely accepted, just like the \log_2 based representation.

All the authors have read and approved the final version of this manuscript.

Sincerely,

A handwritten signature in blue ink, appearing to read 'Gudmundur Hrafn Gudmundsson'.

Gudmundur Hrafn Gudmundsson, PhD

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Iceland.

Thank you for your submission to PeerJ. I am writing to inform you that in my opinion as the Academic Editor for your article, your manuscript "Cyclic mechanical stretch down-regulates cathelicidin antimicrobial peptide expression and activates a pro-inflammatory response in human bronchial epithelial cells." (#2015:09:6825:1:0:REVIEW) requires some minor revisions before we could accept it for publication.

The comments supplied by the reviewers on this revision are pasted below. My comments are as follows:

Editor's comments

I am pleased to inform you that the manuscript is suitable for publication in PeerJ. Before accepting the manuscript officially, please make the final changes suggested by reviewer 1.

Response: We would like to thank the reviewers for their positive reviews on our manuscript. We have discussed with all the co-authors regarding the representation of the q-RT PCR results on a log scale on the Y-axis. Although, the data can be represented on a log₂ scale as suggested by reviewer 1, representing the data as 'relative expression' on Y-axis is also a standard and well accepted method. Please note these published articles where the q-RT PCR data is represented with the term 'relative expression', 'fold difference' or 'fold change' [1) PMID: 20199660, Figure 2B and 2C); 2) PMID: 19939273, Figure 2A and 2B; and an article published in PeerJ where the Y-axis is labeled as Fold Expression, PMID: 23646287, figure 1A-1F)]. We have also added an extra sentence in the figure legends for all the q-RT PCR results, so that the readers are not confused about the method of representation ('Relative expression levels (y-axis) in static untreated cells were defined with an arbitrary value of '1' and changes relative to this value in stretched/treated samples are represented.'). We hope this clarifies the comments from reviewer 1. We have also made other changes suggested by reviewer 1.

If you are willing to undertake these changes, please [submit your revised manuscript](#) (with any rebuttal information*) to the journal within 45 days.

Pankaj Goyal
Academic Editor for PeerJ

Reviewer Comments

Reviewer 1 (Anonymous)

Basic reporting

The manuscript demonstrates that a mechanical stretch down-regulates cathelicidin antimicrobial peptide expression and activates a pro-inflammatory response in the human bronchial epithelial cell line model VA10.

Thanks for critically correcting the manuscript and adding the desired information.

Experimental design

no comments.

Validity of the findings

no comments.

Comments for the author

Just a very few comments:

Lines 197 & 198: it has to be "Miltényi", the 1 afterwards should be removed

Response: We would like to thank the reviewer for pointing out this error. This has now been corrected.

Figures 1-5: the analysis and data interpretation using delta $2^{\Delta\Delta Ct}$ method is absolutely fine, it is a valid and frequently used method, there is no doubt at all! But still you have to mention this on the axis as a figure should be self-understandable for the readership even without the main text. Therefore for axis labelling it should be mentioned that it is a logarithmic calculation with basis 2 as e.g. a fold change of 4 means in the end $2^4 = 16$ fold difference in the RNA amount and not 4 fold difference as you could assume in a decimal system.

Response: The term 'relative expression' refers to the relative expression of target gene with respect to untreated control, where untreated control is represented with an arbitrary value of '1' and changes in treated samples are represented as changes relative to this untreated control (change relative to '1'). Representing the Y-axis with the term 'Relative expression' is a standard practice. In order to label the Y-axis as Fold change (\log_2) we will have to transform all our data to a log scale. This would not add any significant information to our results. Nevertheless, we are aware that q-RT PCR data can be

represented on a log scale and in that case instead of the control untreated sample having the arbitrary value of '1', this sample will have a value of '0' and fold changes that are down-regulated will be represented on a negative scale (commonly referred to as log scale on Y-axis). Thus, we think the method we have used represents data in a standard manner where all the controls have a relative value of '1' and changes relative to this value are represented. Please note these published articles where the q-RT PCR data is represented with the term 'relative expression', 'fold difference' or 'fold change' [1) PMID: 20199660, Figure 2B and 2C); 2) PMID: 19939273, Figure 2A and 2B; and an article published in PeerJ where the Y-axis is labeled as Fold Expression, PMID: 23646287, figure 1A-1F)]. We also used the same Y-axis labeling in our recent published article: PMID:26358366. In all the cases the data on the Y-axis is not log transformed. Thus one of the many ways to represent the q-RT PCR data is to simply label the Y-axis with 'Relative expression' and mention the target gene. This is widely accepted by readers familiar with q-RT PCR data. We have now added in the figure legends 'Relative expression levels (y-axis) in static untreated cells were defined with an arbitrary value of '1' and changes relative to this value in stretched/treated samples are represented.'. We hope this explanation will be sufficient for the reader for clarity.

Reviewer 2 (Anonymous)

Basic reporting

The revised version of manuscript is fine and makes a good scientific reading.

Experimental design

Well planned and executed experiments. The results obtained and reported in the manuscript are suitable for conclusions drawn.

Validity of the findings

Findings of the present study have been duly validated with complementary experiments.

Comments for the author

The overall presentation and scientific content of the manuscript is quite good. The revised version reads much more comprehensive and therefore, it is quite suitable for publication.

Response: We would like to thank the reviewer 2 for the positive comments on the manuscript.

Reviewer 3 (Michael Zasloff)

Basic reporting

I am happy with the revised version

Experimental design

Excellent

Validity of the findings

Important

Comments for the author

An important observation with great potential medical value

Response: We would like to thank Dr. Michael Zasloff for the positive comments on the manuscript.