Author’s response to reviews

Title: The antifibrotic drug pirfenidone inhibits spondyloarthritis fibroblast-like synoviocytes and osteoblasts in vitro

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Version: 3 Date: 26 Aug 2018

Author’s response to reviews:

Dear editor,

Thank you for your careful handling of our manuscript. We really appreciate this. We really believed that the current version was edited as you suggested last time using the wording you suggested. We have again tried to make the corrections suggested as explained in the responses below. We also realize that we missed deleting sentences about myofibroblast differentiation in the last couple of sentences in the Result section, when describing the FCM data and in the beginning of the Discussion section. This was not on purpose. Sorry for the inconvenience.

On behalf of the authors,

Tue

Editor Comments:

I would like you to edit the manuscript carefully. I have repeated the same comments multiple times, but the manuscript has not been revised well. After you have edited the manuscript, please read all of the edited files carefully before sending us.

Major comments;
1. Please show the data of live/dead staining in both control and PFD treated cells in Fig 1C.

Answer: We are showing the Live/dead stain of the stimulated and PFD treated sample. The other conditions are described in the Results section. Otherwise, the figure would be too busy and there were <1% dead cells and no differences as described.

2. It looks like the gating of intact cells are missing in Fig 1C. To gate intact cells, people usually remove FSC-low SSC-low events. To evaluate the proportion of dead cells accurately, the gating of intact cells are required.

Answer: We completely agree. All FCM analyses were performed on intact cells. We have added the gate in Fig 1C.

3. This is the third time for me to tell you the same comments. The interpretation of the results in this manuscript is that PFD suppressed proliferation and cytokine production of SpA fibroblasts and mineralization of osteoblasts, but did not alter the differentiation of fibroblasts to myofibroblasts. Please correct all of the related statement in both result and discussion sections. You should not state that PFD inhibited the expression of aSMA or HLA-DR in the result section, since they are not statistically significant. If you think the no differences are due to small sample size, please increase the number of samples.

Answer: We completely agree that the differences are not statistically significant. Therefore, we also only reported that there was a decrease in all cultures without a significant change. However, we are happy to make the corrections below.

Please correct following parts;

3-1; Abstract, result section; "PFD further showed a modest and non-significant suppression of,," is not correct statement. Based on the statistical analysis, you cannot state that PFD showed a modest suppression. I would recommend to state that PFD did not suppress TGFb-induced upregulation of aSMA,..

Answer: We do not believe that claiming that PFD does NOT decrease aSMA and HLADR is entirely correct either based on the tendency. We have chosen the more neutral statement that there is no significant change.

3-2; Results second paragraph; same above. Also, you should not write that no statistical significant differences are because of large inter-donor variations in result section, since there are no data to support the statement. You cannot deny the possibility that PFD did not suppress the expression of aSMA and HLA-DR. Based on your data, you should understand that you did not detect any effect of of PFD on the expression of aSMA, HLA-DR and ICAM1.
You can discuss that PFD might have mild effect to suppress aSMA and HLR-DR expression in discussion section, but it is only in discussion.

Answer: Again, we have chosen the more neutral statement that there is no significant change in the Result section. We have mentioned that there could be a moderate effect in the Discussion. We removed all remarks about inter-donor variation.

3-3; Results second paragraph; Please remove "PFD seems to decrease TGFb-induced myofibroblast differentiation and IFNg upregulation of HLA-DR molecules in SpA FLS". This is incorrect statement. Please add correct statement instead. If you still believe and would like to state that PFD have some effects on myofibroblast differentiation, you should show the data. It is easy to increase the number of samples, isn't it?

Answer: We agree. We unfortunately missed deleting this sentence in the previous version.

3-4. Discussion first paragraph; What does "myofibroblast formation" mean? The effects of PFD on fibroblasts are cell proliferation and cytokine production.

Answer: We agree. We unfortunately missed deleting this sentence in the previous version.

3-5. Discussion 4th paragraph; Same with 3-1 and 3-2.

Answer: We have mentioned that there could be a moderate effect in the Discussion as you suggested. We removed all remarks about inter-donor variation.

3-6. Discussion 5th paragraph, 3rd sentence; Your data are not consistent with the results of ref 38. Please amend the statement, and discuss appropriately.

Answer: We agree and have only stated that there could be a modest effect in the same way as for aSMA.

Minor comments;

1. Abstract, result section: Please replace <3 of 12 cytokines or chemokines> to <IGFBP3, MCP3 and YKP-40>, and state that this is the results of membrane-based antibody array.

Answer: We agree that this is more precise. However, there was not enough words allowed for these changes in the Abstract. We have made the changes in the Result section.

2. In fig 3C, it is hard to understand the values from 2.5 to 20 of Y axis for MCP-1.
Answer: It is almost impossible to demonstrate the differences between 1-2.5 and 5-50 on the same figure. We have now tried with three segments of the y-axis.