Author’s response to reviews

Title: Investigating the effects of Pirfenidone on TGFβ1-stimulated Non-SMAD signaling pathways in Dupuytren’s disease-derived fibroblasts

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To,
The Editor
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Dear Editor,

Thanks for the recent note regarding our recent manuscript submission titled “Investigating the Effects of Pirfenidone on TGFβ1-Stimulated Non-SMAD Signaling Pathways in Dupuytren’s Disease-Derived Fibroblasts” (Manuscript no. BMSD-D-18-01179R2). We want to thank the Reviewer 1 for his comments in improving the manuscript substantially. As the Reviewer has stated that most of his suggestions have already been addressed in the manuscript, we have made all the efforts to make sure that all of his suggestions are addressed. We have provided point-by-point response to the reviewer’s comments and we have revised the manuscript accordingly. We have made every effort to clarify the queries raised by the reviewer with our response being italicized following the reviewer’s comments.
The authors have substantially improved the data interpretation in this manuscript. My only additional, discretionary revisions pertain specifically to the results and discussion sections. In all figures (1-4), the format is a comparison between CT and DD cells, treated with TGFß-1 and/or PFD. I suggest that the authors address five basic points in these sections.

1) Are the baseline phosphorylation (activation) levels of these enzymes (AKT, ERK1/2, p38) and substrate (MLC2) different between CT and DD?

We have set the “no treatment” as 1 for CT- and DD-derived fibroblasts and then compared it to other treatment conditions. We made this change based on the suggestions you have provided on the first revision of this manuscript. Due to this type of comparisons, we could not determine the baseline activation levels of the different enzymes and substrate that was chosen to study. In the first version of this manuscript, our comparisons were set to no treatment CT-fibroblasts as 1 which was then compared to the other treatments in CT- and DD- cells. With this analysis setup, we found that basal expression of p-ERK, p-AKT and p-MLC was increased in DD-cells except for p-p38.

2) does TGFß-1 treatment significantly alter these levels within groups (CT and DD)?

An increase in the expression levels of p-AKT, p-p38, and p-MLC was seen when DD-cells were stimulated with TGF-beta1, but statistically significant increase was not seen. In CT-cells a statistically significant increase in the levels of p-AKT was seen on TGF-beta1 stimulation. We are reasoning this to the small sample size (n=3) which limited the statistical power and the limitation of this study.

3) does PFD significantly alter these levels within groups?

A statistically significant decrease in p-p38 levels was noted in both CT- and DD-cells after treating with PFD. Though a decrease was noted in other molecules when treated with PFD a statistically significant alteration was not observed.

4) does PFD significantly impact the effects of TGFß-1 treatments within groups?
PFD significantly inhibited the TGF-beta1 mediated increase in p-AKT, p-P38, p-ERK and p-MLC especially in DD-cells. In CT-cells PFD significantly inhibited TGF-beta1-induced p-AKT and p-ERK1/2.

5) did they discern any effects of TGFß-1 or PFD (or both) that are specific to either CT or DD cells?

Interestingly, TGF-beta1-induced levels of all the four molecules were significantly inhibited by PFD in DD-cells focused in this study was significantly reduced in DD-cells when compared to CT-cells.

Some of these points are already addressed in the current manuscript, however some are not, and they would appear to the key discussion points. Perhaps most importantly, if the baseline levels of these molecules in DD and CT cells are not statistically different before treatment (Q1 above), and if PFD has essentially the same effects on both CT and DD cells, does this potentially undermine its utility as a therapeutic for DD without, or with minimal, impact on normal (CT) cellular function? The inclusion of these points in the discussion would substantially enhance the manuscript.

As the Reviewers have stated we have addressed his comments in the manuscript. The other points that the Reviewer has pointed out have been addressed below.

Previous studies have used primary cultures from various tissues to test the effects of PFD, e.g., normal dermal fibroblasts (Lab Invest 2018 May;98(5):640-655), normal lung fibroblasts (Life Sci. 2008 Jan 16;82 (3-4):210-217), normal tenon fibroblasts (J Ocul Pharmacol Ther. 2017 Jun;33:366-374) to name a few. And the conclusions authors have drawn was PFD can potentially inhibit the pro-fibrotic effects elicited by TGF-β1. When the authors tested the effects of PFD on Idiopathic lung-derived fibroblasts and compared to normal lung fibroblasts, the effects of PFD was seen in both cell lines (Life Sci.2008 Jan 16; 82(3-4):210-217), but this did not preclude using PFD as a therapeutic agent for Idiopathic pulmonary fibrosis. In the present study, it would have been much more appropriate to have included fibroblasts derived from unaffected palmar fascial tissue from the DD patients as a control to determine if PFD exerted a similar effect as it did on DD-cells. If PFD could inhibit the pro-fibrotic effects of TGF-β1 on unaffected palmar fascial cells, the findings would add value to PFD’s ability as a prophylactic drug to prevent the progression of the disease. We are basing this from our previous published studies where we have shown that transcriptomic signature of fibroblasts was highly similar between unaffected and affected palmar fascia tissue derived from DD patients (BMC Med Genomics.2012 May 4;5:15). In our opinion, the effects of PFD seen on both CT- and DD- cells should not undermine its ability for its utility as a therapeutic agent for DD.
We hope we have provided a satisfactory response to the reviewer’s queries and we again thank the reviewer for his time and productive comments.

Thanking you,

Sincerely yours,

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