Reviewer's report

Title: Investigating the effects of Pirfenidone on TGFbeta1-stimulated Non-SMAD signaling pathways in Dupuytren's disease-derived fibroblasts

Version: 1 Date: 23 Oct 2018

Reviewer: David O'Gorman

Reviewer's report:

Overview: This submission addresses the effects of Pirfenidone on TGF-ß1-stimulated, non-canonical signaling pathways in primary fibroblasts derived from fibrotic (Dupuytren disease) and phenotypically normal palmar fascia as controls. The findings indicate that, in the presence or absence of TGF-ß1 treatment, Pirfenidone decreases the phosphorylation (and by extension, the activation) of several TGF-ß1-stimulated non-canonical signaling pathways in both DD and CT fibroblasts. These findings could have relevance to the potential for using Pirfenidone as a novel treatment or adjunct treatment for patients with Dupuytren disease-associated palmar digital contractures.

Major typos/edits required: In the results section, the authors make several claims that are not clearly substantiated by the data. A non-significant trend cannot be considered equivalent to a statistically significant difference if the study is appropriately powered. This reviewer wondered if the authors were confusing the effects of PFD and TGF-ß1 treatments within groups (CT, DD), which are often significant, with between group comparisons (CT vs DD), which do not appear to have been assessed in most, if not all, figures. All of these interpretations and statistical analyses need to be clarified and/or corrected in the manuscript.

Specifically, they claim to show "the basal phosphorylation levels of AKT, ERK1/2, and MLC were elevated in DD-derived fibroblasts compared to CT-derived fibroblasts", yet the statistics for the densitometry in the figures pertaining to these data (Figs 1, 2 and 4 respectively) do not indicate any statistically significant differences between the no treatment (Ntx) readings for CT and DD fibroblasts. The authors claim that "TGF-ß1 further increased the phosphorylation levels of the above proteins in both CT- and DD-derived fibroblasts", yet the statistics for the densitometry in the corresponding figures do not indicate any statistically significant differences between the no treatment (Ntx) readings and the TGF-ß1 treatments for CT and DD fibroblasts. The authors claim that Pirfenidone (PFD) "inhibited both basal and TGF-ß1-induced phosphorylation of AKT, ERK1/2, and p38 in both CT- and DD derived fibroblasts". In contrast, their data show no indication that PFD treatment altered ERK1/2 phosphorylation in basal DD fibroblasts, nor that p38 phosphorylation was altered by PFD treatment alone in CT or DD fibroblasts under basal conditions.
Minor typos/edits: There is substantial pixilation in the images of the immunoblots in Fig 2, whereas the other immunoblotting images are of relatively good quality. The authors are requested to provide better quality images in Fig. 2 if that is possible.

The lines in the densitometry graphic indicating statistical significance between treatments were discontinuous in the version of Fig 4 I received, making it difficult to discern which treatments were being compared. Please edit this figure accordingly.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
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No

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