Reviewer's report

Title: Developing an Animal Model of Dupuytren's Disease by Orthotopic Transplantation of Human Fibroblasts into Athymic Rat

Version: 2 Date: 22 December 2014

Reviewer: Megan L Killian

Reviewer's report:

Major Compulsory Revisions:

Statistical analyses do not utilize reliable/validated statistical programs. Additionally, the comparisons made are unclear in the methods text and this section should be revised. Are the authors comparing in vitro qRTPCR results to in vivo qRT PCR results? Additionally, a two-sample t-test is an inappropriate test to use for comparing the luminescence data. We suggest using a repeated measures ANOVA, since the same animals are used throughout the duration of the experiment.

The use of rat skin, as opposed to rat tendon or fascia (which are the comparable cells by which the DD and CT fibroblasts were derived), for validating qRT-PCR results as human-specific should be discussed as a limitation. Validation assays should also be performed to ensure that the rat skin samples, which the authors show do not express the genes of interest using human-specific probes, do indeed express comparable rat-specific genes (in other words, validate that the rat skin samples were not damaged or degraded, e.g., due to differences in processing of tissue). Likewise, the authors should address the contributions of expression from native rat cells in the rat forepaw tissues, and screen for similar genes using rat-specific probes.

Clarity needs to be made defining exactly what tissues were removed from the qRT-PCR assays. Additionally, rationale for the time point selected needs to be made. Given the transient nature of gene transcription, a single time point and lengthy duration from original insult may not be enough to clearly tell the story the authors are hoping to project.

Sample size for each experiment should be noted in the methods (e.g., how many samples went to histology? qRT-PCR?). Were the transplants unilateral or bilateral? How were the DD- and CT-cells separated for analysis? The methods read as follows: 6 total rats were used. In each rat, labeled DD- and CT-cells were injected into the forepaw, and assays were done on the entire tissue. In the results and figures, it is more clear that the DD-cells were injected in one forepaw and the CT-cells were injected into another. The methods should be modified for clarity here.

How were samples used for both RT-PCR and histology? If separate animals were used, the sample size for each experiment should be noted. If the same
animals were used for both qRT-PCR and histology, this should also be clarified. From the figures, I have gathered that N=4 animals were used for qRT-PCR, leaving N=2 animals for histological outcomes.

Intensity of stain using Masson's trichrome as an outcome for histology is misleading, given the potential variability in section thickness and stain duration. It could also be differences in brightness during imaging or the location selected on the slide. Additionally, if the analysis is based on intensity of blue staining alone, it appears, from the representative images, that Figure 3A has darker blue stain than Figure 3B, yet the authors make no mention of this and focus primarily on the differences between control and DD-injected samples. The authors should consider having a certified pathologist or blinded reviewer(s) score the sections for inflammation, vascularity, and foreign bodies.

RQ needs to be defined, as it's an abbreviation that is used in the figures but not defined in the text anywhere. Additionally, the authors must define what the difference is between CT-derived fibroblasts and 1x10E7 CT-derived fibroblasts is. It would be more clear to use text such as "cultured cells" vs. "implanted cells."

Minor Essential revisions:

Comparisons between cultured cells and implanted cells should be made with caution, especially if the in vitro cells were not maintained in culture for the duration of time of cell implantation. In vivo environments will most definitely alter viability, proliferation, and expression of genes compared to in vitro environments, especially for 2D culture, and the duration of cell culture also alters expression.

Some of the wording in the text can be improved. For example, the results are somewhat awkward; Line 272 begins with "Results are shown in Figure 4." Results of what? The sentence does not stand on its own. Use of words like "dramatic" in the results should also be removed. If the differences are statistically significant, then the authors can say as such, but "dramatic" or "dramatically" are biased assessments and should be removed from the results section.

Figure 2 needs revision such that the scale bar is (1) accurate across all images and (2) legible. The text and resolution too small as presently represented.

In the Figure 4 legend, this sentence is confusing: "Values represent the mean ± SEM of two independent experiments on RNA derived from the tissues of four animals performed in triplicate." What do the authors mean by "two independent experiments on RNA derived from tissues"?

Appropriate normalization of luminescence data should be clarified. Can the authors compare luminescence on day 7 to that on day 63?

Rationale for using paraffin-embedded histological sections vs. frozen sections should be made. With frozen sections, the authors could have imaged for DiR-positive cells. However, paraffin embedding likely damages the lipophilic
membrane, and as a consequence, the DiR stain is removed. It would be beneficial to have histological sections that support the luminescence data.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I have no declarations.