Reviewer’s report

Title: Gene expression markers of tendon fibroblasts in normal and diseased tissue compared to monolayer and three dimensional culture systems

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Reviewer: Graham Riley

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The question posed is well defined and the paper is generally well written.

The methods are generally well described although the following points - specific minor essential revisions - need addressing:

1) The authors admirably use GeNorm and Normfinder to assess the most stable reference genes, although it is subsequently unclear which reference genes are used to standardise levels of gene expression in the results. Please state if only a single reference gene was used for the data, and if so which one. See also my point 6 below.

2) Following from this point, were the significant differences in gene expression maintained (ie still significant) regardless of which reference gene was used?

3) The methods are unclear as to how long 2D cell cultures were maintained before cells were harvested. I note that 3D cultures were maintained for 5 days after the addition of 10% serum.

4) Following this point, was any work undertaken to assess changes in gene expression with time in culture. After all, the cells will be 'settling down', and in the case of the 3D cultures, there will be the establishment of tensile forces through the gel by the contractile activities of the cells. These could have effects on the mRNA levels measured, particularly of mechanosensitive genes such as Tenascin-C.

5) The introduction and figures mention (and show) 11 genes, but in actual fact it appears that 12 genes were analysed. For some reason, Tenomodulin is not shown on Figure 1 - why is this gene not shown? It would be potentially useful to observe how relative levels of expression compared to the other genes (although I am aware that it is not a truly quantitative comparison). Unless there is good reason not to show it, I think it should be shown on the figure 1.

6) With respect to the reference genes, I would prefer that the genes SDHA and ACTB are given their full names. Methodologically, I would also like to know how many different potential reference genes were assessed. Since this paper is reporting a potentially useful dataset that would enable others to make informed choices about the appropriate genes to use as markers or reference genes, it would be beneficial to add a figure showing the full normalisation dataset and the genes that were included for comparison, and how variable they were.
Results and figures
Apart from the points raised above, I noticed the following minor points which need addressing/changing:

1) Figure labels and legends refer to COLA2 instead of COL1A2
2) The X axis legend in figure 1B refers to COLXA1 - this should be COL10A1 as referred to elsewhere
3) The labelling of both figure 1 and 2 has gone awry. Graphs are incorrectly labelled and should be correctly designated (ie a) and b) have been swapped. It would also help if either lower case or upper case were used (ie A or a, B or b). Please make sure that in the results the correct graphs are referred to.
4) The expression levels of COL1A2 are reported to be higher in foetal tissues compared to mature tendon (line 278), but this difference is not drawn on the figure (Fig 2B - incorrectly shown as Fig 2A - see point above). I note that the significant difference in tenascin levels is shown.
5) Please use the same nomenclature throughout when referring to skeletally immature tendon and yearling tendon. In the methods, this term is used to describe both foetal and yearling tendon. The graph in Fig 2B uses the phrase 'immature tendon' when it might have been more appropriate to use 'yearling tendon'

Discussion
I think it is stretching the point to declare that 'this study has confirmed that a panel of marker genes are required to identify tendon cell phenotype'. After all, only a total of 12 genes were assessed, and only two genes were originally proposed as potentially specific markers of tendon cells - tenomodulin and scleraxis. These were shown not to be useful on their own. I am still slightly puzzled why tenomodulin was excluded when COL1A2 was included, as this was able to discriminate between tendon and cartilage. Tenascin C did not discriminate between tendon and cartilage, yet this was included. It is still theoretically possible that a genome wide screen could identify specific markers. This study has actually shown that the existing choice of genes are of little use on their own.

The discussion is otherwise well balanced, placed into context and the limitations of the work are adequately discussed. I think it slightly disappointing that no data from stretching the tendon cells was included and it would be interesting to know if the application of strain was able to modulate tenascin C and scleraxis expression. Clearly there is much more work to be done in this area and this is appropriately acknowledged by the authors.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I am a consultant for Wyeth Laboratories on its tendinopathy research programme. I also am active in this research area and have been conducting similar studies on human tendon tissues and cells.