Reviewer’s report

Title: Cellular pharmacodynamic effects of Pycnogenol® in patients with severe osteoarthritis: a randomized controlled pilot study

Version: 1 Date: 25 Apr 2017

Reviewer: Stefan Toegel

Reviewer’s report:

The authors have adequately responded to most of my comments. I still think that this is an interesting study that qualifies for publication, however, some issues remain to be addressed, as listed below.

*) In their revision, the authors argue that "Due to the very low amount of tissue obtained from all patients and therefore the very low amount of cells and RNA isolated from the tissue, we had decided to check the RNA quantity and purity (A260/A280 ratio) for further processing and not the RNA integrity via agarose gels stained with ethidium bromide". Besides the fact that there are more advanced methods for the quality control of RNA preparations (i.e., Bioanalyzer), which need only tiny amounts of RNA, I wonder about the low RNA yield mentioned by the authors. The Methods section (page 8) clearly states: "The complete residual articular cartilage was removed from the patients' samples". Enzymatic digestion of the entire articular cartilage obtained during total knee replacement surgery should yield several millions of viable chondrocytes. This is also confirmed by the authors stating that "Cell aliquots of approximately 1 x 106 cells were shock-frozen in liquid nitrogen and stored at -80 °C. Only cell samples showing > 95 % viability were used for further experiments". Such a cell yield would result in sufficient amounts of RNA to perform any required quality control procedures. Is there a technical problem with RNA isolation procedure in the lab?

*) The authors' ambition to follow the MIQE guidelines is truly appreciated! However, I still have concerns regarding the authors' approach with reference gene selection/validation. I agree that the use of 3 reference genes is recommended, but of course all of the selected reference genes must be "stably" expressed to allow normalization. I do not see the usefulness of including an unstable reference gene for normalization of qPCR results, and I do not believe that the MIQE guideline intend to give this recommendation. I also do not see the benefit for the reader to present all calculations with 3 reference genes (including"unstable" SDHA) and then to exclude SDHA for recalculation. This is rather part of assay validation that each lab should perform before publishing the most robust results. The notion that SDHA is "unstable" might qualify for a technical note (maybe in the Methods section or in Supplementary files) but, in my eyes, it is not valid to foreground this aspect in the final paper. In my view, the authors have two options. Either they find a third stable reference gene and present a novel set of recalculated results, or they simply use the 2 most stable (already identified) reference genes which would also fairly comply with the MIQE requirements. In any case, this will improve the manuscript with regard to clarity.
There is also a second remark related to this issue: The authors should clearly describe their approach for reference gene stability assessment. Which parameter was used to identify "stability", what was the cut-off value? Without such a description, the methodology leaves the impression of arbitrary decisions. Maybe geNorm would be a better option as this tool (besides stability rankings) also provides suggestions on the optimal number of reference genes required for reliable normalization of qPCR data.

*) Similarly, when two patients were defined as outliers regarding pre-established criteria, it would be more straightforward to exclude associated data from calculations. At present, the presentation of results (particularly on page 12, line 280-300) is rather confusing.

*) The manuscript should include a clear description of the origin of the Pycnogenol capsules (company,…).

*) line 50: remove "at".

*) line 171: Life Technologies (capital letters)

*) "osteoarthritis" is not consistently abbreviated as OA within the manuscript.

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

No

**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?**
If not, please explain in your comments to the authors.

Yes

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