Author's response to reviews

Title: Human telomerase reverse transcriptase and glucose-regulated protein 78 increase the life span of articular chondrocytes and their repair potential

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Author's response to reviews: see over
Dear Dr Blanco and Dr Quiniquini,

We herewith resubmit our manuscript entitled “Human telomerase reverse transcriptase and glucose-regulated protein 78 increase the lifespan of articular chondrocytes and their repair potential” for publication as a research article in *BMC Musculoskeletal Disorders*. Thank you for your email and for the opportunity to respond to your reviewers’ recommendations. We have performed some new experiments, made methodological modifications, and included some further explanations as the reviewers requested. We have also carefully revised the manuscript in accordance with the recommendations of the reviewers. Our specific responses to the reviewers’ comments are given below. We believe that our findings and their significance will be of interest to your readers.

Thank you in advance for considering our revised manuscript for publication in *BMC Musculoskeletal Disorders*.

Yours sincerely,

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Reviewer: Jose Luis Fernandez

-Minor Essential Revisions:
Introduction: “As cells age, their chromosomes gradually become shorter as a result of mitosis”. It should be clearer: In proliferative cells, telomeres from chromosomes gradually became shorter as a result of the DNA replication end problem. A brief explanation of telomeres and telomere biology should be necessary.

We have made the change you suggest, and have added a brief explanation of telomeres and telomere biology to the Background, as follows. “Telomerase is a ribonucleoprotein that enzymatically adds DNA sequence repeats (TTAGGG) to the 3' ends of DNA strands in the telomeric regions at the ends of chromosomes.” I have also added the following explanation to the Background, as follows. “The telomerase allows for replacement of short bits of DNA known as telomeres, which are otherwise shortened when a cell divides via mitosis. In normal circumstances, without the presence of telomerase, if a cell divides recursively, at some point all the progeny will reach their Hayflick limit. With the presence of telomerase, each dividing cell can replace the lost bit of DNA, and any single cell can then divide unbounded. While this unbounded growth property has excited many researchers, caution is warranted in exploiting this property, as exactly this same unbounded growth is a crucial step in enabling cancerous growth.”

Although it seems that the different parameters determined in the study are similarly improved in both young and old chondrocytes, a clear comparison is only showed for proliferative capacity and for type II collagen RNA production. It should be specified if there were differences between young and old untransfected and transfected chondrocytes, in cell density in 3D cultures, DNA content, glycosaminoglycan production and repair capacity of osteochondral defects.

You suggest that it is difficult to interpret our results because the controls were a mixture of young and old chondrocytes. I have altered the results, basing them on the old chondrocyte control, except in Figure 1.

It must be discussed why was GRP78 selected for transfection. Is there a deficiency of this enzymatic activity in chondrocytes? What exactly means ER stress and how could it be present in chondrocytes? Does aging influence this
ER stress?

I have added the following explanation to the Discussion.

“ER stress can alter protein synthesis in cells [38]. One mechanism by which ER stress promotes apoptosis in cells is by driving the accumulation of structurally abnormal proteins [39], which are ordinarily repaired by ER chaperones to prevent age-related cell death. GRP78 is an example of a chaperone protein that regulates protein folding in the ER and thus contributes to cell survival [40].”


Was quantification of glycosaminoglycans related to DNA concentration, as suggested in Figure 3? This is not clear in Materials and Methods.
The ‘GAG content’ refers to the corrected quantification of glycosaminoglycans, or the amount of GAG per µg of DNA.

A Q-PCR should be preferable for RNA quantification of type II collagen.

We performed a real-time PCR analysis of type II collagen, according to the reviewer’s suggestion, and have described the results in the revised manuscript.

Tables 1 and 2 are indicated for OA score, but they are not present in the manuscript. It is suspected that the higher the score, the lower the damage?

I sincerely apologize for the omission of Tables 1 and 2 from the first manuscript submitted.

I have added Tables 1 and 2 to the revised manuscript.

Discuss why hTERT increases proliferation doublings, their increase depending on age of chondrocytes, but not achieving immortalization. Are telomeres progressively decreasing with cell replication in spite of telomerase activity? Is telomerase activity decreasing with progressive cell cycles?

According to the literature, the ectopic expression of the hTERT gene in neural
progenitor cells induces telomerase activity, stabilizes telomeres, and extends their replicative lifespans. We could not determine the immortalization of the chondrocytes in this study. Chondrocytes are differentiated somatic cells and not stem cells, and we speculated that because they have the lowest telomerase activity in nature, these chondrocytes could not be immortalized.


Aged cells are known to contain a higher frequency of numerical and structural chromosome aberrations and DNA mutations. It may be a great concern to immortalize or propagate these cells and to introduce them in a living organism. Possible long-term effects should be discussed. We recognize that chondrocytes from aged patients have poor proliferative activity, and addressed this problem in regenerative cartilage therapy using autologous cells. The present study demonstrates that the focal gene delivery to aged articular chondrocytes causes strong repair activity and may be therapeutically useful for the regeneration of the articular cartilage. However, as the reviewer notes, aged cells are known to contain higher frequencies of numerical and structural chromosome aberrations and DNA mutations. Further research will be required to determine whether the method outlined in this study can overcome this problem.
Reviewer: Ali Mobasheri

Specific Comments:
Figure 1. This graph needs to be improved. Each of the cell populations will need to be represented using different colours or symbols. The current presentation of Figure 1 may confuse some readers.
I have altered Figure 1 according to the reviewer’s suggestion.

Figure 4. The data presented in this figure is neither, clear nor convincing. The approach used by the authors seems very outdated. They should have performed quantitative PCR instead. New data needs to be presented here, either using quantitative PCR or a combination of quantitative PCR and western blotting for type II collagen.
We performed a real-time PCR analysis of type II collagen in response to the reviewer’s suggestion.

Figure 6. This reviewer is unable to follow the histopathology scoring of the OA cartilage samples and the statistical evaluation used. This part of the manuscript is rather weak and needs to be more robust and more convincing.
I sincerely apologize for the omission of Tables 1 and 2 from the first manuscript submitted.
I have added Tables 1 and 2 to the revised manuscript.