Review of: "Synovial Fluid Mitochondrial DNA Concentration Reflects the Degree of Cartilage Damage After Naturally Occurring Articular Injury"

Annette McCoy[1]

1 University of Illinois at Urbana-Champaign

Potential competing interests: The author(s) declared that no potential competing interests exist.

Summary: This study presents a series of *in vitro*, *ex vivo*, and *in vivo* experiments all aimed at documenting the release of mitochondrial DNA (mtDNA) by chondrocytes/cartilage explants/joints under conditions of inflammatory and mechanical stress. Extracellular mtDNA was detected in culture media after exposing chondrocytes to inflammatory stress via IL-1b and after mechanically injuring bovine explants. Further, mtDNA was measurable in synovial fluid after experimental and naturally-occurring joint injury *in vivo*. The authors contend that mtDNA, released as a result of mitochondrial dysfunction secondary to an injury, may be an early biomarker that could indicate the onset of early PTOA. However, the work shown could not determine whether mtDNA is merely a consequence of early disease or a contributor to disease progression. They also suggest that mitoprotective agents such as SS-31 may represent a novel therapeutic modality for PTOA.

General Comments: The authors are to be commended for such a comprehensive set of experiments, ranging from cell culture to naturally occurring disease, and they provide compelling evidence to support their main conclusion that mtDNA could be a biomarker of early PTOA. Their conclusions regarding the efficacy of the mitoprotective agent SS-31 are less well-supported, though intriguing. Specific questions follow:

1. A significant amount of biological variability was noted in the *in vitro* chondrocyte work, but this was not commented on for the other experiments. This variability was significant enough that a normalization approach was applied – but again, this was not repeated in the other experiments. What was the reasoning behind this different approach?

2. The normalized mtDNA:nDNA ratio for the *in vitro* chondrocytes was significantly different between stimulated and control cells at 12 hours, but not at 24 hours. Do you have an explanation for this finding? How does it relate to the more prolonged mtDNA release noted in the other experiments?

3. Are there any potentially relevant species differences (equine vs bovine vs murine) that could have

implications for the findings of the various experiments?

4. For the explant experiment, why not have a comparison group of non-impacted, treated explants? It's hard to know whether the SS-31 was actually addressing mitochondrial dysfunction related to the impact trauma, or time-related changes in mitochondrial function. In Figure 2, it appears that there was a trend towards increased mtDNA release in the injured untreated explants, although it did not reach statistical significance. Was there any post hoc testing performed on these data to determine if sample size was an issue?

5. The timing of treatment with SS-31 was either 1h or 12h post-injury. Please comment on the clinical relevance of this timing.

6. The functional experiments examining the mechanism of action of SS-31 were presumably done in healthy/unstimulated chondrocytes. Do the findings hold true when the chondrocytes are stressed (e.g. by IL-1b)?

7. The findings in the experimental *in vivo* study seem to suggest protection against cell death rather than specifically against release of mtDNA. This distinction could be commented on in the discussion.

8. For the injured clinical patients, how long after the presumptive injury occurred were they radiographed, and was this at the same time as synovial fluid sampling? It would be interesting to see how this correlates with mtDNA measurements. We know that radiographic changes are delayed, so if synovial fluid was collected relatively soon after injury it is completely unsurprising that radiographic changes would be minimal. It is unfortunate that the control group is so different from the injured group, as this makes comparisons a bit suspect.

9. You suggest that mtDNA may be predictive of post-operative outcomes, but were other factors (such as age, sex, pre-injury performance level, size and location of lesion, etc.) taken into account in this evaluation?

10. In the discussion, you state that synovial fluid mtDNA may be a less invasive diagnostic method. Are you proposing this as an alternative to arthroscopic evaluation? As these horses need to have their chips removed, what would be the advantage of this? It would be interesting to see how arthroscopic findings correlated with number of race starts (or other performance parameters); were these more or less predictive than the mtDNA concentration? If mtDNA is to be useful as a diagnostic marker, it would be important to know how it changes over time after a naturally occurring injury, rather than the single time point presented here.