Testing Whether Sex-Specific Senescence in the Lizard, *Anolis sagrei*, Translates to the Cellular Level

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Within numerous species, males and females age at different rates resulting in varying susceptibility to age-related diseases. This sexual dimorphism is understood at the organismal level and the trend is that females live longer than males[1]. Our group has found this to be true in the lizard, *Anolis sagrei*, posing this species to be a potential model for studying the basis of sex-specific aging. Seeking to investigate this phenomena on the cellular level, cell culture provides a model to study senescence (the deterioration and loss of function leading to cell death) in a controlled environment. Biomarkers of aging are commonly used to quantify the rate of senescence. Telomeres, found at the ends of chromosomes, are the most commonly used biomarker of aging[2,3]. Upon each genome replication during cell division, telomere length shortens; thus, the length generally shortens with age. Telomere maintenance varies with sex and age in mammals[4]. This study aims to further develop brown anoles as a model for sex-specific aging by (1) establishing cell lines from young lizards, (2) establishing cell lines from old male and female individuals, and (3) testing if telomere length is a good predictor of senescence and sex-specific senescence in these lizards.

The fibroblast cell culture was set up using tail tissue from three-year-old adult males (n = 3), three-year-old adult females (n = 3), and one-month-old hatchling males (n = 3; n_{tot}= 9). At each passage, cells were collected and divided between seeding the next plate, being frozen for long-term storage, and being frozen for analysis. Through this study, we have demonstrated it is possible to establish cultures from this species at the beginning and end of their lifespan (3-4 years in the wild). Culturing methods are continually being optimized for these non-model organisms, so our practices are working to fill a gap in knowledge. The cells frozen for long-term storage are the start of a biobank for future research in our lab. From each sample group, snap-frozen cells from passage two were used in telomere analysis.

Telomere length of the nine cell lines were quantified through DNA isolation, DNA quantification, then quantitative polymerase chain reaction (qPCR)[5,6,7]. We found that on average, telomere length in cells lines started from young lizards were longer than in cell lines started from adults (average starting quantity of 19193 vs 13265), and cell lines from adult female lizards had longer telomeres than adult males at the same (old) age (average starting quantity of 14404 vs 13265). Although not statistically significant at p = 0.05, which is perhaps because of our small sample size in each group, these results are consistent with the hypothesis that telomere dynamics in lizards are similar to mammals in that the telomeres decrease with age and old females have longer telomeres than males of an equivalent age. Viewed cautiously, these findings provide preliminary support for telomeres as a viable biomarker for senescence and sex-specific senescence in this lizard species. Future work will increase the number of cell lines for each age group for further telomere analysis along with other common biomarkers of aging.

In conclusion we have been able to establish fibroblast cell lines from young and old anole lizards that can be used for future experiments, and we have preliminary support for telomeres being a biomarker of senescence in these reptiles. These data and resources further support further study into using the brown anole as a promising model to study sex-specific aging.

Statement of Research Advisor

The field of aging biology has limited vertebrate models to study sex-specific aging. Milica and her PhD student mentor, Amanda Clark, have established the first known set of age-specific cell lines in a reptile species that can be used for future experiments in the context of aging. Furthermore, we have very limited understanding of telomere dynamics in reptiles as a whole, and this is the first study to quantify telomeres in anole lizards. These accomplishments are major

steps forward in using reptiles as models of aging.
– *Tonia Schwartz, Biological Sciences*

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