Henry

Journal of Ophthalmology & Optometry

Research Article

Evidence of RPE Cell Senescence in AMD from Comparison of Telomere Lengths in Central and Peripheral Retina

Kozlowski MR[1]*, Kozlowski RE[1] and Spear J[2]

[1]*Arizona College of Optometry, Glendale, AZ, USA*

[2]*Pearle Vision, Everett, WA, USA*

Abstract

Senescence of Retinal Pigment Epithelial (RPE) cells has been proposed as contributing to the pathology of Age-related Macular Degeneration (AMD). The present study measures the Terminal Restriction Fragment (TRF) lengths of RPE cells from both AMD affected and control retinas of aged donors as an indicator of cell senescence. In an ARPE-19 cell line, cells that were approaching senescence were found to have TRF lengths of 3.10bp. RPE cells from the AMD affected retinas had similarly short TRF lengths, suggesting that they also were near or at senescence. In addition RPE cell TRF lengths from the macular regions of the retinas affected by AMD were significantly shorter than those from their peripheral regions, consistent with the greater pathological effect of AMD in the macular region. RPE cells from control retinas also had short TRF lengths, but no significant difference in length was found between macular and peripheral regions. Thus, the present results are consistent with a role of RPE cell senescence in the pathology of AMD, but point to the need for additional corroborative data.

Keywords: Cell senescence; Macular degeneration; Retinal pigment epithelium; Telomere

***Corresponding author:** Kozlowski MR, Midwestern University, 19555 N59th Avenue, Glendale, AZ 85308, USA, E-mail: mkozlo@midwestern.edu

Received Date: August 27, 2020

Accepted Date: September 16, 2020

Published Date: September 21, 2020

Citation: Kozlowski MR, Kozlowski RE, Spear J (2020) Evidence of RPE Cell Senescence in AMD from Comparison of Telomere Lengths in Central and Peripheral Retina. J Ophthal Opto 2: 005.

Introduction

Age related Macular Degeneration (AMD) is one of the main causes of vision loss worldwide [1]. In the United States alone, 10% of all individuals between 65 and 75 years of age, and 30% of those over 75 years of age, have a visual impairment related to AMD [2]. The global prevalence of AMD is estimated to be 196 million, and this number is expected to rise to 288 million by 2040 due to population aging [1]. Senescence of Retinal Pigment Epithelial (RPE) cells is one of the biochemical mechanisms that have been proposed as contributing to the pathology of AMD [3-5].

Cell senescence is a naturally occurring process that changes the structural and functional characteristics of cells in ways which can be harmful to the tissue that they comprise [6]. These changes have been implicated in the pathology of several disorders including osteoarthritis, atherosclerosis, and benign prostatic hyperplasia [6-8]. Although the etiology of cell senescence is multifaceted, one of its major causes is critical shortening of chromosomal telomeres [6,9]. Telomeres are specialized regions at the ends of chromosomes that associate with a complex of proteins to prevent the chromosome ends from being mis-identified as double-stranded breaks [6,10]. When telomeres become critically shortened, they are unable to form these complexes. The chromosome ends are then treated by the cell as persistent double-stranded breaks, which triggers cell senescence.

Shortening of telomeres generally occurs in one of two ways [11]. First, shortening can occur in actively dividing cells through the loss of short pieces of DNA at the ends of chromosomes during each round of replication. Second, it can occur as a result of DNA damage from environmental insults, such as oxidative stress, which causes the loss of long fragments of telomeric DNA [6,10,12]. Because cell divisions and cell damage accumulate over time, both of these processes are likely to contribute to the telomere shortening that has been reported to occur with aging [11]. This makes senescent cells, and the disorders to which they contribute, more prevalent in older individuals.

If RPE cell senescence contributes to AMD, then certain conditions should apply. First, the telomere lengths of RPE cells in retinas affected by AMD should be sufficiently short to be consistent with the presence of cell senescence. This should be particularly true in the central region, which contains the macula, since the macula is more affected by this disorder [13,14]. Some studies with animal models of AMD, which also show greater RPE damage centrally, suggest that this damage due to oxidative stress [15]. Second, the RPE cell telomere lengths in the central region of retinas affected by AMD should also be shorter than those in the peripheral region, which is less effected by the disorder [13,14]. Finally, there should be a more significant shortening of telomeres in central region of retinas affected by AMD than of those unaffected by this disorder. The present study assesses the telomere lengths of RPE cells from the central and peripheral regions of both retinas affected by AMD and those unaffected by this disorder to determine whether these conditions are met and, thereby, the hypothesis that RPE cell senescence contributes to AMD is supported.

Materials and Methods

Human RPE cells

Human eyes were obtained postmortem from 11 donors over the age of 70 years (range=71-92 years of age; one eye per donor) from

the Anatomic Gift Foundation (Hanover, MD). The medical records of 6 donors contained a diagnosis of AMD from a licensed medical doctor whereas the other 5 had not been diagnosed with any ocular disease except cataracts. The ages of donors with AMD (average=85) and donors with no retinal disease (average=81) were not significantly different (two-tailed t-test, p=0.187). The donor eyes were removed within 24 hours after death, placed on ice, and shipped to our laboratory overnight. The retinas with the attached choroid and RPE layers were dissected from the eyes within 24 hours of arrival at the laboratory. The tissue was divided into a central region, which was taken to be the area lying within a circle 1 cm in diameter centered on the fovea, and a peripheral region, comprising the remaining tissue. The RPE layer was then separated from the retinal and choroidal layers in both the central (central RPE) and the peripheral samples (peripheral RPE) and frozen until DNA extraction (1-4 days).

Cell culture

ARPE-19 cells (CRL-2302; non-transformed) were thawed and cultured according the instructions provided by the supplier (ATCC). Briefly, the cells were quickly thawed and grown in T-25 cell culture flasks in 5 ml of DMEM: F12 culture medium supplemented with 10% FBS. The cells were re-fed after 3-4 days. After one week of growth in the flask, the cells were released with Trypsin-EDTA and counted using a Moxi-Z™ cell counter (Orflo Technologies; Hailey, ID). The Moxi Viability Index™ was used as an indicator of cell viability, and all cultures had a viability index of at least 90%. One tenth of the cells were then transferred into a new flask and diluted to the original volume with fresh medium. The culturing process was then repeated. The population doubling level (PDL) was calculated as equal to 3.32 x (log Xe - log Xb) + S, where Xb is the number of cells seeded, Xe is the cell number at the end of the growth period (i.e. before splitting), and S is the starting PDL.

Measurement of terminal restriction fragment length

Genomic DNA was isolated from the APRE-19 cells or dissociated RPE cell layers using a Cyclo-Prep™ genomic DNA purification kit (VWR, Radnor, PA). The DNA was then concentrated using a DNA Clean and Concentrator™-5 kit (Abcam, Cambridge, MA). Terminal Restriction Fragments (TRF) were prepared and sized on agarose gels using the TeloTAGGG telomere length assay kit (Fisher Scientific, Pittsburgh, PA). The blots from the agarose gels were scanned with an Alpha Imager (Protein Simple, Santa Clara, CA), and the bands were analyzed with the GelAnalyzer™ software package (www.gelanalyzer.com). TRF lengths were calculated as specified in the TeloTAGGG kit instructions.

Statistical analysis

Individual means were compared using t-tests. When multiple t-tests were performed on the same data set, the probabilities were adjusted using the False Discovery Rate (FDR) method of Benjamini and Hochberg [16].

Results and Discussion

In the present study, relative telomere lengths were assessed by measuring the TRF lengths of genomic DNA. This approach is well accepted for comparing telomere lengths and its results agree with those of other quantitative measurement techniques [17]. In order to estimate the TRF length associated with RPE cell senescence, ARPE-19 cells were cultured through a sufficient number of population doublings bringing them nearly to the PDL level at which senescence occurs. Although some controversy exists over the use of these cells as a surrogate for native human RPE cells, we and others have shown that ARPE-19 cells display many of the attributes of native RPE cell if used prior to the onset of senescence, which occurs between 59 and 77 population doublings [18-20]. After this point, they also display many of the signs generally seen in senescent cells including the appearance of a senescent-like cell morphology, increased staining for senescence-associated beta-galactosidase activity, and an attenuation in the rate of telomere shortening [18]. In addition, cultured RPE cells of other types also show both telomere shortening and the cellular characteristics of senescence when induced to senesce prematurely by oxidative stress [5].

RPE cells were also obtained from the eyes of human donors of greater than 70 years of age. Some of these donors had been diagnosed with AMD, and others had no diagnoses of ocular disorders other than cataracts. Because the length of time between the death of the donor and isolation of RPE cell DNA differed between donors, and could be up to 6 days long, we investigated whether this delay had an effect on the measurement of TRF length. When TRF lengths were plotted as a function of interval between donor death and DNA isolation we found that only a miniscule reduction in TRF length was associated with the increased delay in its measurement (Figure 1). This supports the reliability of our TRF length measurements, and agrees with other reports of the stability of postmortem DNA [21].

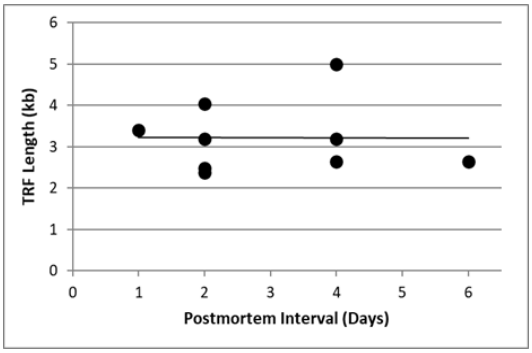

Figure 1: Effect of postmortem delay in DNA extraction and purification on measured TRF length. Values show the average of central and peripheral TRF measurements in kilobases (kb). The line representing a linear fit of the data is nearly horizontal ($y = 0.0065x+3.24$; $R^2 = 0.0001$) demonstrating very little relationship between TRF lengths measured and postmortem delay.

ARPE-19 cells that were approaching senescence (PDL's 51 to 55) were found to have TRF lengths averaging 3.10kb (Table 1). This agrees with our previous findings, and is similar to the results obtained by others with primary RPE cells [5,18]. This TRF length is shorter than those for ARPE-19 cells at lower PDL's. For example, ARPE-19 cells at PDL 24 have a TRF length of 4.16kb [18]. The TRF lengths of RPE samples from the central areas of retinas affected by AMD were very similar to those of the pre-senescent ARPE-19 cells (Table 1). Although ARPE-19 cells are only a surrogate for native RPE cells, as discussed above, and there were differences between the culturing and assaying of these two types of cells, this result suggests that cell senescence was at least imminent, if not already present, in the native RPE cells. Interestingly, the TRF lengths of RPE cells from the central areas of control eyes were also similar to those for pre-senescent ARPE-19 cells, and were, in fact, slightly shorter than those from AMD affected eyes. A parallel difference, however, was seen in the RPE cells from the peripheral areas, which are less affected by AMD [13,14]. This suggests that differences in TRF lengths between the AMD affected and control retinas were more related to variations in telomere dynamics between the donors than to AMD pathology.

Sample	TRF Length (kb)	N
ARPE-19 - PDL 51.0 - 55.0	3.10 ± 0.17	4
AMD Retina - Macular	3.25 ±1.10	6
AMD Retinal - Peripheral	3.57 ±1.06	6
Shortening in Macular vs. Peripheral	0.22 ± 0.21*	6
Control Retina - Macular	2.80 ± 0.53	5
Control Retinal - Peripheral	3.08 ± 0.62	5
Shortening in Macular vs. Peripheral	0.28 ± 0.53ǂ	5

Table 1: TRF lengths of DNA isolated from ARPE-19 cells approaching cell senescence and from the retinas of donor eyes. Donor eyes had either been diagnosed with AMD or found free of retinal disease (Control). Values for TRF length are given in kilobases (kb) and represent means plus or minus standard deviations. The number of combined samples is shown under "N".

*Macular region significantly shorter than peripheral region, one-tailed paired t-test, $p < 0.05$

ǂMacular region not significantly shorter than peripheral region, one-tailed paired t-test, $p=0.68$

In AMD affected retinas, there was a reduction of TRF lengths in the central retina compared to the peripheral retina and this reduction was statistically significant (Table 1). The individual reductions in central relative versus peripheral TRF lengths in control and AMD retinas are compared in figure 2. What is notable is not the average amount of reduction, which was similar between the two groups, but the consistency of the reduction in the AMD retinas. The TRF lengths of 5 of the 6 central samples from the AMD group were at least 100 base pairs (bp) shorter than the peripheral samples, and even the sixth sample had a small reduction (Figure 2). The finding that the TRF lengths of RPE cells in the central region were uniformly and significantly shorter than those in the periphery in retinas affected by AMD suggests that the central RPE cells had a more senescent profile. In the control retinas, only 2 of 5 central regions had RPE cell TRF lengths that were at least 100bp shorter than their peripheral samples, and one had a TRF length 750 bp greater than the peripheral sample from the same retina (Figure 2). Thus, although the control retinas had an average reduction in RPE cell TRF lengths between the central and peripheral regions similar to that in the AMD retinas, the reduction was not consistent and, consequently, not statistically significant (Table 1). Lack of a significant difference in telomere lengths between RPE cells from central and peripheral has also been reported by others using a somewhat broader age range of samples [22].

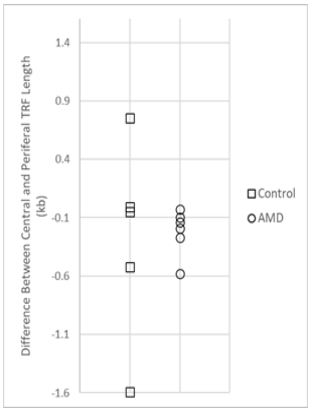

Figure 2: Differences between TRF lengths in central and peripheral retina from retinas affected by AMD and from control retinas. Negative values indicate a shorter length centrally. Lengths are as in Figure 1.

Conclusions

The present study shows that RPE cells in the central region of retinas affected by AMD have TRF lengths that are in the range found ARPE-19 cells that are nearing senescence. This suggests that these RPE cells are at least approaching cell senescence. The TRF lengths of RPE cells from the central regions of control retinas were, however, also of a similar length to those of APRE-19 cells approaching senescence. Thus, the approach of RPE senescence based on a short TRF length does not appear to be a sufficient condition for the occurrence of AMD. This may be because short TRF lengths do not indicate when RPE cells have actually crossed the threshold to senescence. Thus, in the present study, the relative lengths of central and peripheral TRF's were compared to determine whether RPE cells from the central retinal in AMD eye were at least closer to senescence than those from control eyes based on having relatively shorter TRF lengths.

Although RPE cells from the central region of control retinas appeared to have shorter TRF lengths than those from the peripheral region, this difference was neither consistent nor statistically significant. In fact, one the five samples had a longer TRF length centrally and in two others the reduction was minimal (less than 100bp). On the other hand, in the AMD retinas, central RPE cells had TRF lengths that were consistently shorter than those from peripheral retina. Thus, although the average shortening was of the same magnitude as in the control retinas, it was seen in all six samples, was greater than 100 bp in five of the six, and was statistically significant.

In summary, two of the three conditions set forth earlier in this report for supporting the contribution of RPE cell senescence to AMD (i.e. short RPE cell telomeres in retinas affected by AMD and shorter telomeres in the central areas of retinas affected by AMD than in the peripheral areas) appear to have been met. The third condition, a more significant shortening of RPE cell telomeres in the central region of retinas affect by AMD than in control retinas, also appears to have been met since there was a consistent, and statistically significant, relative shortening of RPE TRF lengths in central compared to peripheral retina in AMD retinas but not in control retinas. Thus, the present results generally support a role for RPE cell senescence in AMD.

There are, however, at least two caveats to this interpretation. The first is the possibility that both the shortening of telomeres in central retinal RPE cells and the pathology of AMD may occur in response to other aging or lifestyle related disorders, but that the telomere shortening does not contribute to the pathology of the AMD. The likelihood of this is tempered by the many connections that have been found between the physiology of cell senescence and the pathology of AMD [3]. The second is that, given the relatively modest shortening of TRF lengths in central areas of AMD retinas compared to peripheral areas and the small sample size of this study, the present results may not be generalizable. Both of these qualifications point to the need for additional corroborative data.

Acknowledgements

This work was supported by a Research Stimulation Grant from Midwestern University.

References

1. Seddon JM (2017) Macular Degeneration Epidemiology: Nature-Nurture, Lifestyle Factors, Genetic Risk, and Gene-Environment Interactions - The Weisenfeld Award Lecture. Invest Ophthalmol Vis Sci 58: 6513-6528.

2. Kanski JJ (2017) Age-related macular degeneration. Clinical ophthalmology: A systematic approach. New York: Elsevier.

3. Kozlowski MR (2012) RPE cell senescence: A key contributor to age-related macular degeneration. Med Hypotheses 78: 505-510.

4. Blasiak J, Pawlowska E, Szczepanska J, Kaarniranta K (2019) Interplay between Autophagy and the Ubiquitin-Proteasome System and Its Role in the Pathogenesis of Age-Related Macular Degeneration. Int J Mol Sci 20: 210.

5. Matsunaga H, Handa JT, Aotaki-Keen A, Sherwood SW, West MD, et al. (1999) Beta-galactosidase histochemistry and telomere loss in senescent retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 40: 197-202

6. Campisi J (2013) Aging, Cellular Senescence, and Cancer. Annu Rev Physiol 75: 685-705.

7. Rodier F, Campisi J (2011) Four faces of cellular senescence. J Cell Biol 192: 547-556.

8. Burton DGA (2009) Cellular senescence, ageing and disease. Age (Dordr) 31: 1-9.

9. Bernadotte A, Mikhelson VM, Spivak IM (2016) Markers of cellular senescence. Telomere shortening as a marker of cellular senescence. Aging (Albany NY) 8: 3-11.

10. Harley CB, Futcher AB, Greider CW (1990) Telomeres shorten during ageing of human fibroblasts. Nature 345: 458-460.

11. Ghimire S, Hill CV, Sy FS, Rodriguez R (2019) Decline in telomere length by age and effect modification by gender, allostatic load and comorbidities in National Health and Nutrition Examination Survey (1999-2002). PLoS One 14: 0221690.

12. Victorelli S, Passos JF (2017) Telomeres and Cell Senescence - Size Matters Not. E Bio Medicine 21: 14-20.

13. Friberg TR, Domalpally A, Danis R, Clemons TE, Sadda SR, et al. (2016) Peripheral Retinal Abnormalities Associated with Age-Related Macular Degeneration in the Age-Related Eye Diseases Study 2 (AREDS2). Investigative ophthalmology & visual science 57: 12.

14. Sunness JA, Massof RW, Johnson MA, Finkelstein D, Fine SL (1985) Peripheral Retinal Function in Age-Related Macular Degeneration. Arch Ophthalmol 103: 811-816.

15. Tisi A, Flati V, Monache SD, Lozzi L, Passacantando M, et al. (2020) Nanoceria Particles Are an Eligible Candidate to Prevent Age-Related Macular Degeneration by Inhibiting Retinal Pigment Epithelium Cell Death and Autophagy Alterations. Cells 9: 1617.

16. Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J Roy Stat Soc B Met 57: 289-300.

17. Lai TP, Wright WE, Shay JW (2018) Comparison of telomere length measurement methods. Philosophical transactions of the Royal Society of London Series B, Biological sciences 373: 1741

18. Kozlowski MR (2015) The ARPE-19 cell line: Mortality status and utility in macular degeneration research. Curr Eye Res 40: 501-509.

19. Dunn KC, Aotaki-Keen AE, Putkey FR, Hjelmeland LM (1996) ARPE-19, a human retinal pigment epithelial cell line with differentiated properties. Exp Eye Res 62: 155-169.

20. Kozlowski MR, Putnam N, Kozlowski RE, Baker J (2018) Cumulative effects of low intensity blue light exposure on the functioning of cultured RPE cells. Annual meeting of the American Academy of Optometry.

21. Shved N, Haas C, Papageorgopoulou C, Akguel G, Paulsen K, et al. (2014) Post Mortem DNA Degradation of Human Tissue Experimentally Mummified in Salt. PLoS One 9: 110753.

22. Desgarnier MCD, Zinflou C, Mallet JD, Gendron SP, Méthot SJ, et al. (2016) Telomere Length Measurement in Different Ocular Structures: A Potential Implication in Corneal Endothelium Pathogenesis. Invest Ophthalmol Vis Sci 57: 5547-5555.

Henry Journal of Acupuncture & Traditional Medicine

Henry Journal of Anesthesia & Perioperative Management

Henry Journal of Aquaculture and Technical Development

Henry Journal of Cardiology & Cardiovascular Medicine

Henry Journal of Case Reports & Imaging

Henry Journal of Cell & Molecular Biology

Henry Journal of Tissue Biology & Cytology

Henry Journal of Clinical, Experimental and Cosmetic Dermatology

Henry Journal of Diabetes & Metabolic Syndrome

Henry Journal of Emergency Medicine, Trauma & Surgical Care

Henry Journal of Haematology & Hemotherapy

Henry Journal of Immunology & Immunotherapy

Henry Journal of Nanoscience, Nanomedicine & Nanobiology

Henry Journal of Nutrition & Food Science

Henry Journal of Obesity & Body Weight

Henry Journal of Cellular & Molecular Oncology

Henry Journal of Ophthalmology & Optometry

Henry Journal of Perinatology & Pediatrics

Henry Publishing Groups, Glanmire, Cork, Republic of Ireland, T45 KD66
Tel: +353 98010312; E-mail: contact@henrypublishinggroups.org
https://www.henrypublishinggroups.com/