Qualification and Quantification of Telomeric Elongation Due to Electromagnetic Resonance Exposure

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Abstract. High frequency electromagnetic resonance (EMR) due to electromagnetic fields (EMFs) can directly affect intercellular molecular mechanisms. Formerly thought to have no direct impact on biological tissue an increasing amount of evidence supports that EMFs can alter the way that cells transcribe proteins, regulate cellular maintenance or enter/bypass senescence. Repeated electromagnetic field shock (REMFS) will up regulate the HSR/HSF1 pathway in young cells within mice delaying senescence directly leading to upkeep in tissue maintenance compared against control mice. In this particular case the REMFS act as an environmental stressor activating a cell signaling cascade elucidates a response within the cell that ultimately up-regulates repair and maintenance systems (Perez, 2008). One particular protein generally involved in maintenance and repair is telomerase reverse transcriptase (*h*TERT). Due to the end replication problem of semi-conservative replication of DNA each time a cell divides approximately 50-100 base pairs are lost from the ends of chromosomes. In order to prevent loss of important genetic sequence, DNA incorporates molecular "aglets" called telomeres that are simply a short hexo-nucleotide sequence of "TTAGGG" which can only be restored via *h*TERT. However, *h*TERT is only minimally active in mature mammalian cells to a continual loss of telomeric DNA in cells of adult organisms, senescence of those cells and ultimately leads to the phenomenon aging (Weaver, 2008). If aging is simply the manifestation of short telomeres then the restoration of telomeric sequence should have the reverse effect. Activation of *h*TERT in aged telomerase deficient mice indicates chromosomal telomere elongation leads to increased cellular proliferation, organ upkeep and performance and an overall physiologically "younger" appearing mouse (Jaskelioff, 2010). My colleague, Dr Norm Shealy of Holos University, has worked with EMFs as alternative medical treatment since the early seventies and has utilized several devices in his practice to treat an array of ailments from depression to cancer. One such instrument consists of an electric spark tester and a copper conductor that when current is applied emits oscillating EMFs with frequencies between 54 and 78 GHz. We speculated that this particular range of frequencies possibly acts upon either some aspect of the SUMOlynation pathway or the ubiquitin pathway associated with telomerase as human studies, performed by Dr. Shealy, indicate a consistent increase in telomere length of leukocyte DNA. In order to qualify this claim a series of primary cell lines isolated from *Mus musculus* (lab mouse) were subjected to this oscillating frequency for 30 minutes a day for approximately 4 months and telomeric regions were measured via Monochromatic Singleplex QPCR. The results appeared to indicate telomere maintenance and subtle elongation. A second set of tissue cultures are undergoing the identical treatment and will be subjected to quantification of telomere length via Southern Blot to be compared against negative controls.This will allow relatively fast assessment of the amount of telomere elongation or loss with treatment when compared to the expected loss of telomeres over a given amount of time.

Introduction

Most mammalian somatic cells will divide and eventually enter a senescent state. However, stem-cells retain their ability to divide almost perpetually due to their ability to maintain their telomeres. Telomeres are repetitive sequence that occur at the ends of chromosomes and act as molecular aglets to protect important regions of DNA. During replication a small portion of telomeric DNA is lost and when telomeres become heavily eroded the cell will enter senescence. The activation of telomerase, a telomere building protein, in somatic cells in mice leads to an increase in organ upkeep and even for cells to

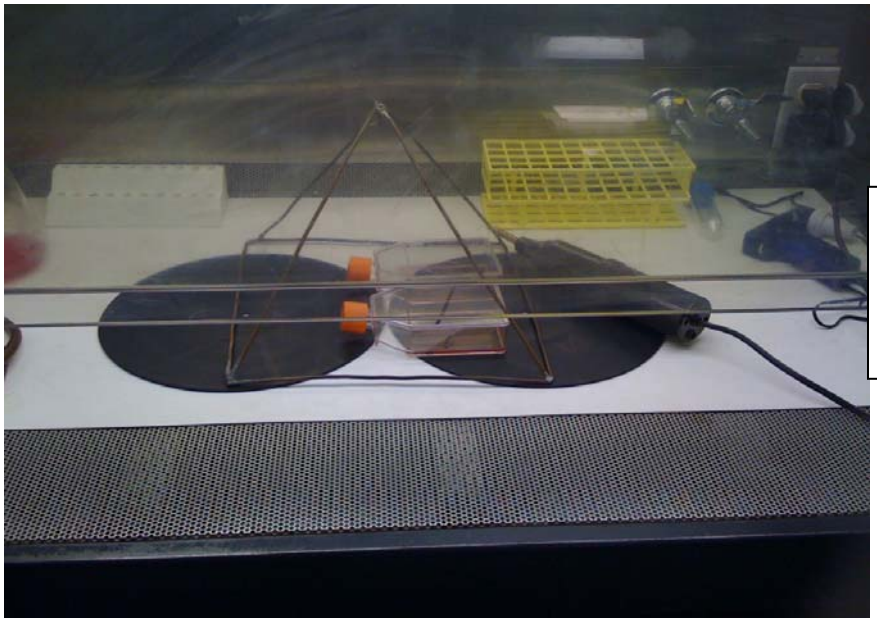
revert from senescence to an actively dividing state. In addition, electromagnetic field stimulation of cell lines *in vitro* stimulates cell signaling pathways that lead to an array of transcriptional activation. If telomerase can be activated via electromagnetic resonance exposure within the cell it could lead to an increase in cellular upkeep without the administration of drugs.

Aim

To first qualify then quantify changes in telomeric length in cell lines exposed to a specific range of electromagnetic resonance between 54 and 78 GHz at 50 to 75 decibels for 30 minutes a day and compare against cell lines from the same original culture. Cell passages occur every two to three weeks at which time genomic DNA samples are taken, quantified via NanDrop and telomere length is measured with singleplex and monochromatic multiplex QPCR and southern blot analysis. Post qualification and quantification telomerase levels will be measured via RTPCR and a possible mechanism will be explored for the activation of telomerase via electromagnetic resonance exposure.

Method…

- Cell lines isolated from *Mus musculus* common lab mouse) using cold trypsinization technique
- Cell lines maintained daily and overall health assessed by visualization with phase contrast optics
- Cell lines treated with electromagnetic resonance for 30 minutes a day under sterile hood, negative control group placed under sterile hood in adjacent lab for 30 minutes a day
- Telomeric lengths assessed using the southern blot technique and singleplex and multiplex monochromatic QPCR



Cell cultures under tissue hood during treatment with electromagnetic resonance

Negative control cell cultures under an adjacent hood during exposure time of experimental cultures

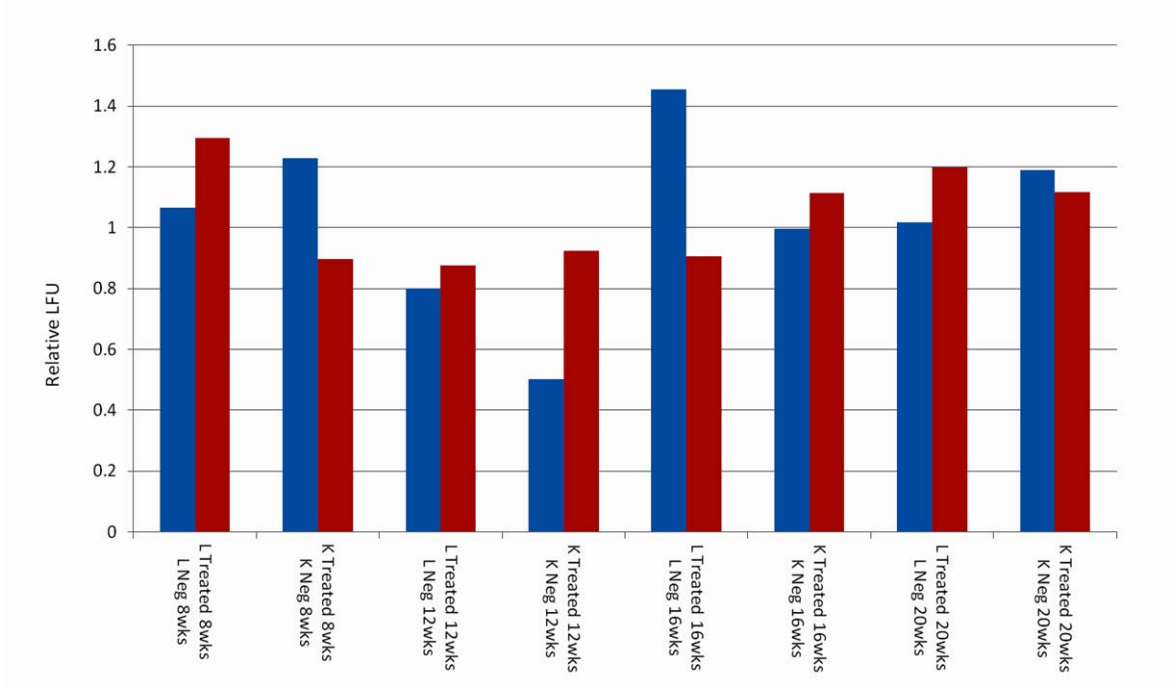
Results

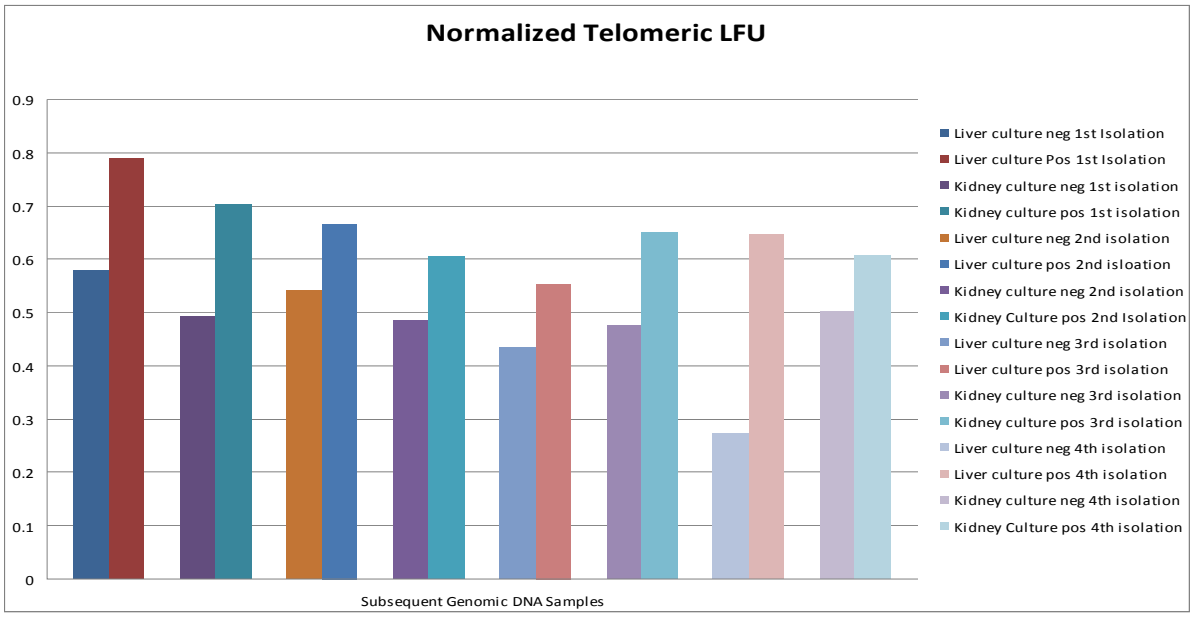
The preliminary work for this project involved treating 2 separate fibroblast cell culture isolated from either a kidney or liver of *Mus musculus.* Singleplex QPCR was used to measure relative telomere length against a single copy gene copy known to occur only one time within the mouse genome and creating a PCR product of a known size. This allows for relative calculation of telomeric repeats against the known size of the single copy gene copy PCR product.

With each cell division, due to the mechanism of DNA polymerization, between 50 and 200 nucleotides are lost from telomeric ends. Fibroblasts in cell culture will divide 2-6 times per passage depending on growth factor concentration and flask volume. Therefore, telomeric regions will experience a gradual decay of 100-1200 nucleotides per cell passage under normal circumstances.

The following graphs illustrate the relative telomere length acquisition from singleplex QPCR. The singleplex technique was performed in duplicate to ensure values remained relatively consistent. This preliminary data was performed to justify continuation of the experiment using larger sample sizes and more sensitive techniques.

The figure on the next page illustrates relative telomere length against single copy gene copy amplicon lengths at DNA isolation intervals. Under normal circumstances, a gradual decline in length would be expected due to telomere decay.



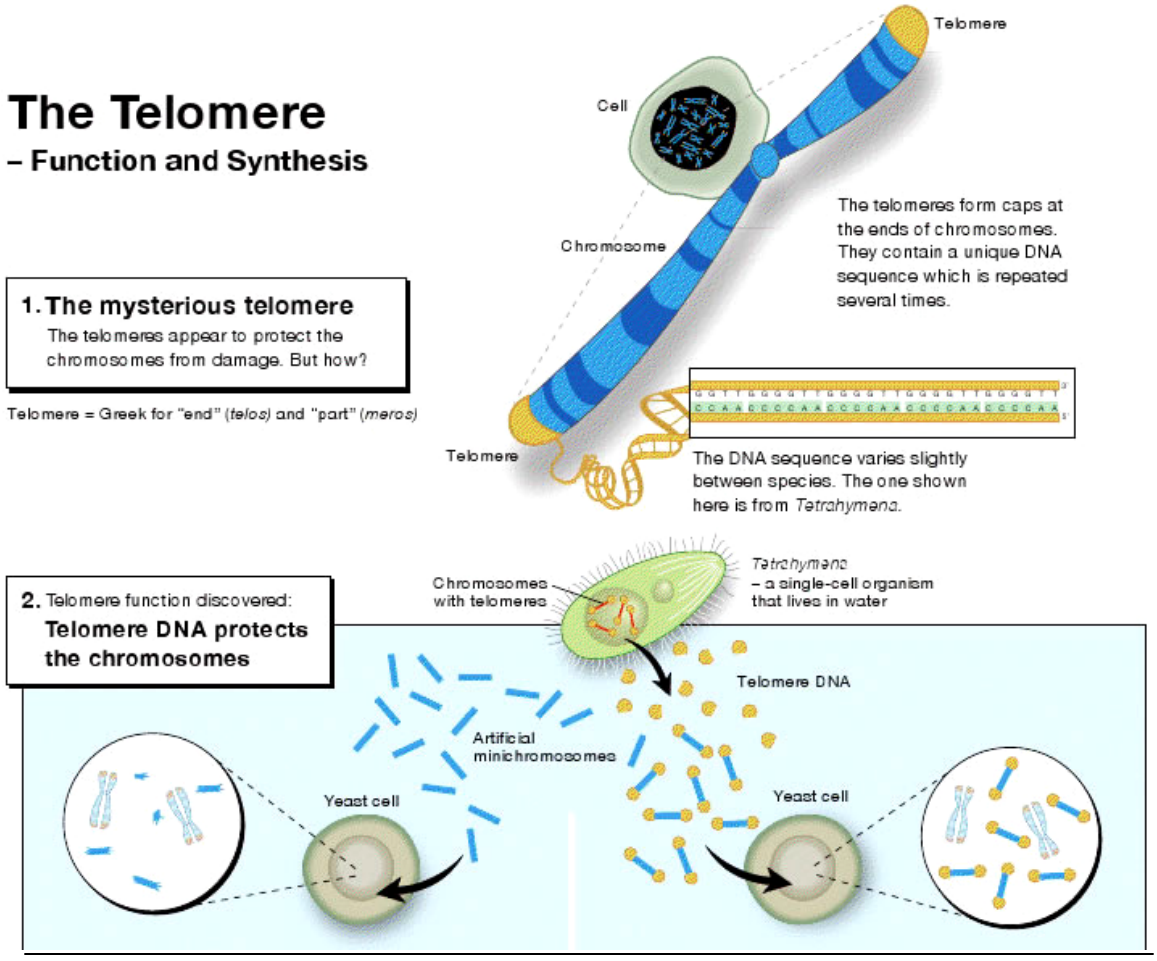


Singleplex QPCR was performed a second time to ensure that telomere decay was indeed halted by electromagnetic resonance exposure

Conclusion

The data from the preliminary experiments appears to support that electromagnetic exposure at this frequency is somehow effecting telomeric maintenance. According to the data generated from the singleplex PCR telomeric lengths are maintained and in some instances elongated. After 20-30 passages in culture telomers normally erode to a critical point that triggers cells to enter senescence. The graphs, however, show a maintenance of telomere length even into relatively old age of the cell cultures.

Unfortunately, in the original experiment negative control cell lines were placed in an adjacent sterile tissue hood within 1 meter of the treated cells. It appears that negative control group may also have been receiving treatment as there telomeres were maintained as well. The sterile tissue hoods used in this experiment are primarily composed of aluminium and stainless steel, both great conductors of electromagnetic fields. Therefore, both hoods may have been acting as large antennae amplifying the electromagnetic signals.



Current and Future Work

Currently, fibroblast cultures originating from mouse kidneys, endothelial cultures originating from mouse blood vessels and astrocytoma cultures originating from human

neural tissue are under investigation for the effects of electromagnetic resonance exposure on telomeric regions of DNA and will be subjected to southern blot analysis and monochromatic multiplex QPCR, both more sensitive techniques than singlex QPCR. In the future telomerase activity will be measured and ultimately a mechanism will be investigated to determine the pathway activated by electromagnetic exposure that elicits the telomeric response.

REFERENCES

Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, Sahin E, Kost-Alimova M, Protopopov A, Cadiñanos J, Horner JW, Maratos-Flier E, Depinho RA. Telomerase Reactivation reverses tissue degeneration in aged telomerase-deficient mice. 2010. *Nature*. Nov;28 (7328) 102-6.

Perez FP, Zhou X, Morisaki J, Ilie J, James T, Jurivich DA. Engineered repeated electromagnetic field shock therapy for cellular senescence and age-related diseases.2008. *Rejuvenation*. Dec;11(6):1049-57.

Weaver, Robert. 2008. Molecular Biology 4th ed. McGraw Hill, Boston MA. Pp. 708-10