Increasing Somatic Telomerase Expression through Inhibition of the SUV39H1/SUV39H2 Methyltransferases using PEGylated Liposomes

By: Trinh M., Moselhi A., Rahman S., Kerr C.

Abstract

Telomeric decay is known to be the primary cause of ageing in mammals, being the direct cause of other known aging mechanisms such as cellular senescence. Cellular immortality is observed in cells containing sufficient levels of the telomerase enzyme complex, which is responsible for lengthening telomeres as they decay via cell division. (1,14,15,17) Expression of the human Telomeric Reverse Transcriptase *(hTERT)*gene is known to directly correlate to the upregulation of the enzyme telomerase, and vice versa for repression of the gene. (5,15,17) In this proposal, the groundwork is laid for a treatment which aims to upregulate expression of the human Telomeric Reverse Transcriptase gene by the means of inhibiting the methyltransferase SUV39H1, which establishes the repressive histone mark H3K9Me3 (8). The histone mark would be impeded by the non-specific methyltransferase inhibitor Chaetocin, using a lipid-based liposomes as a drug delivery carrier, and medical polyethylene glycol (Macrogol) as a drug excipient and potential . This treatment method employs the usage of this liposome combination as the primary method of drug delivery

Keywords: Longevity, Chaetocin, PEGLip, Telomerase, Telomeres, human Telomeric Reverse Transcriptase gene, SUV39H1, SUV39H2

Introduction

Cellular immortality describes the ability of a cell to survive and divide without any limit, due to the absence of telomere shortening. In mammals, this behaviour is observed in cells which upregulate the enzyme Telomerase (7), which is responsible for the rebuilding telomeres as they decay through cell division. Upregulation of telomerase is dependant on the upregulation of the gene *hTERT*, which codes for the Telomeric Reverse Transcriptase (TERT) domain of telomerase, a key telomerase domain for the enzyme's own functionality (5,15)

In somatic cells the *hTERT* gene is found to be epigenetically repressed, being in a state of constitutive heterochromatin throughout an organism's life. A common example of cells which are heavily affected telomere shortening and ageing include epithelial cells. In these cells, *hTERT* is repressed epigenetically (8). Our team aimed to lay down the groundwork for a method of activating the *hTERT* gene via targeted inhibition of specific enzymes known as methyltransferases, using a non-specific inhibitor drug known as Chaetocin.

After reviewing multiple different potential target candidates, we decided that the repressive histone mark H3K9Me3 at sub-telomeric regions was the ideal target for us. This histone mark has been noted to be a marker that is crucial in the repression of *hTERT* gene expression. (8) A key note here is that since H3K9Me3 is a global histone methylation mark for pericentromeric regions and heterochromatin maintenance, multiple different enzymes are responsible for laying down the mark at differing regions of the chromosome. This means that there are specifically only two methyltransferases to deal with in regards to the H3K9Me3 mark

at sub-telomeric regions alone; SUV39H1 and SUV39H2. (19)

The inhibition of these two methyltransferases (SUV39H1/SUV39H2) would be done with a combination of Chaetocin, which acts as an inhibitor of the methyltransferase. (10) Varying dosages of Chaetocin would then be delivered in a new drug delivery tool known as a PEGylated Liposomes (PEGLip). The form of PEGLip's we are using were engineered in late 2018 as a non-toxic/immunogenic drug delivery mechanism for patients. Having passed *in vivo* mice models without causing immune responses and performing drug delivery, this proposal would be one of the first of its kind to implement the new PEGLip model. We chose PEGLip's as a superior drug delivery mechanism as they are less likely to trigger patient immune responses during drug delivery, rather than if we used exogenous material such as a gold nanoparticle.

Hypothesis

We hypothesize that if varying doses of the Chaetocin inhibitor are injected into the nucleus in a targeted manner, various degrees of telomerase expression will subsequently be observed. This observation can be made by directly observing telomeric length *in vitro.*

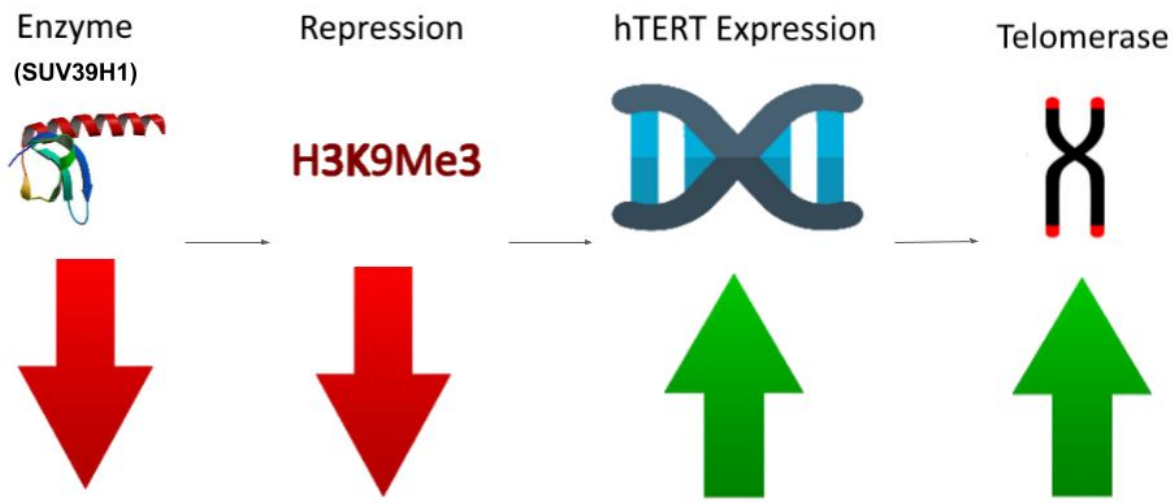


Figure 1) A visual representation of the macro effect our experiment is trying to achieve: Lowering of SUV39H1-induced H3K9Me3 histone methylation at sub-telomeric regions, will allow for more *hTERT* gene expression, which would raise levels of telomerase.

Research Methods

Independent variable: Chaetocin Dosage Levels

Dependent variables: Sub-Telomeric H3K9Me3, Telomere Length

Epidermal Skin Cell Testing

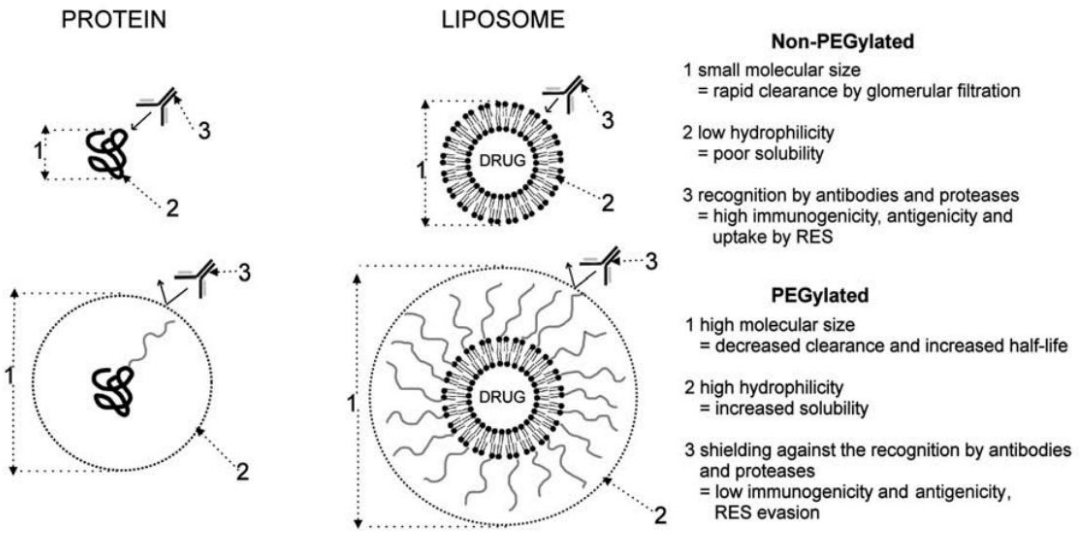
Our experiment would ideally use newly differentiated somatic cells, specifically from tissues which are heavily affected by ageing such as skin epidermal cells. Since somatic skin cells are

plentiful and experience ageing more prevalently relative to other cells, we found this to be the ideal candidate cell type. These cells would be derived from adults aged 18-25, as well as those aged 50-65, in order to fully gage for potentially varying effects of the treatment in patients of different age cohorts. Another possible variable would be ethnicity, which would allow us to observe varying effects of ageing and the treatment on skin cells from patients of different ethnic backgrounds.

We would change Chaetocin concentration in the PEGLip's in order to gauge which levels of Chaetocin are optimal for achieving telomerase expression. After the PEGLip-mediated Chaetocin delivery, we can observe telomere lengths post-injection, to see whether or not the treatment achieved its overall clinical goal of re-growing somatic cell telomeres *in vitro.* In this situation, we would also use a control variable, being blank PEGLip's without any Chaetocin in them.

PEGylated Liposomes Overview

PEGylated liposomes are similar to regular liposomes with the exception of being coated in macrogol, a medical application of polyethylene glycol. PEG's are also FDA approved. An ordinary liposome is a droplet coated in a lipid bilayer which has an empty core in the middle designed for holding a special drug; however, liposomes lack in stability due to how quickly they get processed by the liver. PEG's have more stability and are overall more effective.



The molecule polyethylene glycol helps reduce immunogenicity and antigenic reactions, reduces the clearance time of a drug, increases in-vivo half-life, and improves general pharmacodynamics and pharmacokinetics. It can also deliver oligonucleotides, small drugs, and other biomolecules with local navigation ability. In addition PEG's are naturally water-soluble which allows solid solubility in aqueous solutions.

Medical polyethylene glycol can come in a few forms such as Linear PEG, linear mPEG, and branched mPEG2. PEG is made by linking together ethylene oxide molecules into long chains that extend into linear or branched structures. However, PEG chains shorter than 400Da can be made into a toxic enzyme through alcohol dehydrogenase, and PEG chains lighter than 20kDa will be cleared by renal filtration.

The main differences between the structures mentioned previously are the single function changes. These prevent cross linking errors when trying to merge PEG and another biomolecule. In general, attaching PEG's to any biomolecule will affect key chemical factors such as

hydrophobicity, elastic binding properties, non-bonding chemical changes, and conformation of bond angles which may slightly alter their form. Although PEG-coated liposomes have less biological activity, it compensates by having larger half lifes.

Telomere Length Test with quantitative real-time PCR

Testing for whether or not our optimal outcome (telomere lengthening) has been reached, can be done via quantitative real-time PCR (qPCR). This method allows for the observation of chromosomal telomere length in cells using high-throughput sequencing and could serve as an indicator of the overall efficacy of this treatment model *in vitro.* This method uses oligonucleotide DNA probes to bind to the telomeric sections of the chromosome. The standard oligomer would be 84 bp (14 telomeric DNA hexamer repeats) and the PCR machine would measure the difference of telomeric repeats in comparison to a sample. (20)

The system would create a graph showing fluorescence intensity as a function of the number of cycles completed. The threshold line displays the point at which the fluorescent signal is able to be detected. The cycle threshold (Ct) value is the point at which the function surpasses the threshold line. In the experiment, a standard curve would be created for all of the Ct values as a function of the Log DNA concentration. The unknown DNA concentration could then be found by tying the Ct value with the corresponding Log DNA concentration point on the standard curve.

Additionally, our treatment model targets the primary, but not exclusive methyltransferase behind subtelomeric H3K9Me3, SUV39H1. Although it is likely that SUV39H2 would be

affected given that it differs from SUV39H1 only by the pH of its N-terminus, and that Chaetocin is a nonspecific inhibitor. (10) Nevertheless since SUV39H2 inhibition has not been explicitly stated to occur with Chaetocin, Chaetocin efficacy on SUV39H2 could yield varying degrees of efficacy until either it is proven that Chaetocin can inhibit SUV39H2, or other established inhibitors for SUV39H2 is determined.

Predicted Results

We predict that a significant change in *hTERT* expression will be observed post-treatment in targeted cells. However we cannot say with certainty if obstructing the H3K9Me3 histone mark alone will be enough to induce enough telomerase at levels which the telomeres will regrow at every division.

Future Directions

Our PEGLip-mediated Chaetocin treatment has the potential to cause noticeable changes in *hTERT* expression and subsequent telomerase expression in targeted cells. With that said, we have identified key future steps that will boost the efficacy of this treatment which future research will need to uncover.

One future step will be finding more effective ways of holding the Chaetocin inhibitor drug inside the PEGLip, which is currently being worked on by the researchers that engineered the PEGLip's. Another issue that can be further experimented with is reducing the side effects of polyethylene glycol such as acting like a laxative.

For this specific treatment model it can get costly. On average costs can range from $300-600. To reduce the cost of our model further research must be done to find a cheaper alternative to buying liposome kits as they contribute to the bulk of the cost for our drug delivery solution.

With better drug-containing compatibilities, reduced side effects, and costs, our treatment model can perform at its ideal levels of efficacy and safety. Expanded research in this area will also make it easier to scale this model to a more diverse group of somatic tissues, such as liver cells and kidney cells in the near future.

Works Cited

1. Bodnar, A. G. “Extension of Life-Span by Introduction of Telomerase into Normal Human Cells.” Science, vol. 279, no. 5349, 1998, pp. 349–352., doi:10.1126/science.279.5349.349.
2. Brenner, Charles. “Faculty of 1000 Evaluation for Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors.” F1000 - Post-Publication Peer Review of the Biomedical Literature, 2007, doi:10.3410/f.30699.540812.
3. Byrne, Mark, et al. “Micropatterning Biomimetic Materials for Bioadhesion and Drug Delivery.” Biomimetic Materials And Design, 2002, doi:10.1201/9780203908976.pt3
4. Dreaden, Erik C, et al. “Size Matters: Gold Nanoparticles in Targeted Cancer Drug Delivery.” Therapeutic Delivery, vol. 3, no. 4, 2012, pp. 457–478., doi:10.4155/tde.12.21.

5. Huang, Xinbo, et al. “An Enhanced HTERT Promoter-Driven CRISPR/Cas9 System Selectively Inhibits the Progression of Bladder Cancer Cells.” Molecular BioSystems, vol. 13, no. 9, 2017, pp. 1713–1721., doi:10.1039/c7mb00354d.
6. Issa, Jean-Pierre. “Aging and Epigenetic Drift: a Vicious Cycle.” Journal of Clinical Investigation, vol. 124, no. 1, 2014, pp. 24–29., doi:10.1172/jci69735.
7. Jiang, Xu-Rong, et al. “Telomerase Expression in Human Somatic Cells Does Not Induce Changes Associated with a Transformed Phenotype.” Nature Genetics, vol. 21, no. 1, 1999, pp. 111–114., doi:10.1038/5056.
8. Kim, J., and H. Kim. “Recruitment and Biological Consequences of Histone Modification of H3K27me3 and H3K9me3.” ILAR Journal, vol. 53, no. 3-4, 2012, pp. 232–239., doi:10.1093/ilar.53.3-4.232.
9. Kozielski, Kristen L., et al. “Bioengineered Nanoparticles for SiRNA Delivery.” Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, vol. 5, no. 5, 2013, pp. 449–468., doi:10.1002/wnan.1233.
10. Lai, Y-S, et al. “The SUV39H1 Inhibitor Chaetocin Induces Differentiation and Shows Synergistic Cytotoxicity with Other Epigenetic Drugs in Acute Myeloid Leukemia Cells.” Blood Cancer Journal, vol. 5, no. 5, 2015, doi:10.1038/bcj.2015.37.
11. López-Otín, Carlos, et al. “The Hallmarks of Aging.” Cell, vol. 153, no. 6, 2013, pp. 1194–1217., doi:10.1016/j.cell.2013.05.039.
12. Martucci, Nicola, et al. “Nanoparticle-Based Strategy for Personalized B-Cell Lymphoma Therapy.” International Journal of Nanomedicine, Volume 11, 2016, pp. 6089–6101., doi:10.2147/ijn.s118661.

13. Schmidt, Ryan, and Kathrin Plath. “The Roles of the Reprogramming Factors Oct4, Sox2 and Klf4 in Resetting the Somatic Cell Epigenome during Induced Pluripotent Stem Cell Generation.” Genome Biology, vol. 13, no. 10, 2012, p. 251., doi:10.1186/gb-2012-13-10-251.
14. Takakura, M. “Telomerase Activation by Histone Deacetylase Inhibitor in Normal Cells.” Nucleic Acids Research, vol. 29, no. 14, 2001, pp. 3006–3011., doi:10.1093/nar/29.14.3006.
15. Valls-Bautista, Cristina, et al. “HTERT Methylation Is Necessary but Not Sufficient for Telomerase Activity in Colorectal Cells.” Oncology Letters, vol. 2, no. 6, 2011, pp. 1257–1260., doi:10.3892/ol.2011.386.
16. Zane, Linda, et al. “Common Features of Chromatin in Aging and Cancer: Cause or Coincidence?” Trends in Cell Biology, vol. 24, no. 11, 2014, pp. 686–694., doi:10.1016/j.tcb.2014.07.001.
17. Zhang, Zi-Xiong, et al. “Subtelomeric Demethylation Deregulated HTERT Expression, Telomerase Activity, and Telomere Length in Four Nasopharyngeal Carcinoma Cell Lines.” *Cancer Biotherapy and Radiopharmaceuticals,* vol. 29, no. 7, 2014, pp. 289–294., doi:10.1089/cbr.2013.1581.
18. Lazarovits, James, et al. “Synthesis of Patient-Specific Nanomaterials.” *Nano Letters*, 2018, doi:10.1021/acs.nanolett.8b03434.
19. O’ Caroll, Donal, et al. “Isolation and Characterization of *Suv39h2*, a Second Histone H3 Methyltransferase Gene That Displays Testis-Specific Expression” *Mol Cell Biol*. 2000 Dec; 20(24): 9423–9433.

20. O'callaghan, Nathan J, and Michael Fenech. “A Quantitative PCR Method for Measuring Absolute Telomere Length.” *Biological Procedures Online*, vol. 13, no. 1, 2011, p. 3., doi:10.1186/1480-9222-13-3.

21. Cherblanc, F., Chapman, K., Brown, R., Fuchter, M. “Chaetocin is a nonspecific inhibitor of histone lysine methyltransferases” *Nature Chemical Biology*, 2013, pp. 136–137., doi:10.1038

22. Abdullaeva, Zhypargul. “Synthesis of Nanomaterials by Prokaryotes.” Synthesis of Nanoparticles and Nanomaterials, 2017, pp. 25–54., doi:10.1007/978-3-319-54075-7_2.

23. Paola Milla, Franco Dosio and Luigi Cattel, “ PEGylation of Proteins and Liposomes: a Powerful and Flexible Strategy to Improve the Drug Delivery”, Current Drug Metabolism (2012) 13: 105. https://doi.org/10.2174/138920012798356934