Concept Paper

Toward the Systemic Replacement of Mitochondrial DNA with Somatic Mutations

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Toward the Systemic Replacement Of Mitochondrial Dna With Somatic Mutations

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Abstract:The accumulation of lipofuscin, i.e., indigestible intracellular debris, might be the main cause of age-related diseases that we see today. However, without being able to replace mutated mitochondrial DNA (mtDNA) and damaged and mutated nuclear DNA (nDNA), we will still eventually succumb to aging. Thus, we must save copies of mitochondrial and nuclear DNA at as young an age as possible, or at least from cell types with the lowest rates of mutation. MtDNA has a 10-100x higher rate of mutation than nDNA, as mitochondria are sites of free radical production. We may need to replace mtDNA before damaged and mutated nDNA. If so, we will need a strategy to deliver pristine mtDNA to cells around the the body and destroy the old mtDNA. A strategy for doing so is described herein.

Keywords: mitochondrial DNA mutations; nuclear DNA damage; nuclear DNA mutations; tissue-resident macrophage replacement; secretory autophagy; arrestin domain containing protein 1 [ARRDC1]-mediated microvesicles (ARMMs); Cryptococcus neoformans

Introduction

I recently described an approach for systemically removing lipofuscin [1]. It relies on tissue-resident macrophage (TRM) replacement from edited HSCs that have been transplanted using non-genotoxic conditioning [2]. A similar strategy may enable us to replace mutated mitochondrial DNA (mtDNA) throughout the body. This would be important if mtDNA replacement is necessary prior to the replacement of cells with damaged [3] and mutated [4] nuclear DNA (nDNA) using whole-body induced cell turnover (WICT) [5]. It will likely take a longer period of time to figure out how to enact WICT safely in the human body. As mtDNA mutations accumulate at a 10-100x faster rate than nDNA mutations due to free radical production in the mitochondria, they may have to be addressed prior to the development of WICT.

The only cells that will definitely require mtDNA replacement, if it is necessary before WICT, are stem cells and long-lived, post-mitotic cells. However, a number of non-stem cells in renewing tissues may also have become reversibly senescent as a result of mtDNA mutations. Thus, a non-specific treatment may be of use.

Systemic lipofuscin removal should help to keep mtDNA mutation and nDNA damage and mutation rates to a minimum. Additionally, overexpression of mtDNA repair genes and inhibition of the DREAM complex [6] may help stave off these issues.

However, if mtDNA mutations accumulate too quickly for WICT, we need a way to fundamentally correct them. We must sequence our mitochondrial and nuclear genomes at as early an age as possible. For those who are currently elderly, harvesting DNA from tissues with low mutation rates, e.g., germline stem cells, might be necessary. Mitochondrial DNA heteroplasmy may need to be taken into account, as well.

Strategies for Replacing Somatically Mutated mtDNA:

One way to replace mtDNA throughout the body would be to secrete mitochondria from edited TRMs after systemic TRM replacement using arrestin domain containing protein 1 [ARRDC1]-mediated microvesicles (ARMMs) [7,8]. Self- or trans-replicating RNA [9,10] can also be exported,

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which would encode TFAM to increase mtDNA copy number in target cells [11], as well as nucleases that target old mtDNA. The new mtDNA that is being exported would have mutations in non-coding regions or synonymous or even non-synonymous mutations in coding regions. It was shown that certain SNPs in the mitochondrial DNA are actually beneficial, so this should be possible [12,13].

Importantly, the entire mitochondrial genome based on a digital sequence can be "printed" and utilized therapeutically, as the genome is only 16,569 base pairs and mitochondrial epigenetics would likely not be a concern.

Monocytes appear to frequently secrete extracellular vesicles [14], and TRMs likely can do so as well. However, the secretion of larger vesicles such as microvesicles may be more rare due to plasma membrane size constraints - even when stimulated. The rate of microvesicle secretion may not be rapid enough to make a sizable dent in the overall number of mtDNA copies in target cells. It is also unclear how to substantially increase the availability of donor cell mitochondria for secretion purposes. PGC-1α, TFAM, and Drp1 overexpression could help to maximize mitochondrial biogenesis, mtDNA replication, and fission, but having a fragmented mitochondrial network could be detrimental for TRM functionality or cause inflammation. Endosomal escape may also be an issue with ARMMs, although mitochondria donated by mesenchymal stem cells via ARMMs were able to fuse with recipient cell mitochondria7; fusion with recipient cell plasma membranes may occur8. Furthermore, mitophagy could destroy at least some of the pristine genomes before the mitochondria that contain them fuse with target cell mitochondria and the genomes can be replicated. It was demonstrated that a mitochondrial targeted meganuclease can produce shifts in heteroplasmy over time [15,16]. However, there probably must be a large enough initial quantity of the genome that is to be selected via replicating RNA, which has a limited lifetime. Thus, although each mitochondrion may contain multiple mitochondrial genomes, if only a small number of mitochondria are delivered to a target cell with hundreds or thousands of mitochondria, this may not be enough - i.e., selection for the unmutated mitochondrial genome may take too long to be feasible via replicating RNA. If transfer of a cytoplasmic DNA plasmid [17] can be enacted, wherein it is taken up into the nucleus of target cells [18], low-level selection could potentially be effected for as long as necessary.

Bacteria can conjugate with mammalian mitochondria, although whether second strand synthesis occurs afterwards is unknown [19]. A strategy called Mr PB [20] could be employed to replace TRMs systemically with new versions that contain intracellular bacteria that replicate up a tolerable copy number, at which point they would be restrained via quorum sensing. Mr PB just involves elimination of patient TRMs and repopulation via repeated infusions of immune cells - instead of HSC transplantation. Even if a few copies of a bacterial vector can be delivered to target cells, there is a range of mitochondria morphology that is possible in terms of fusion and fission. If the bacteria conjugate with a large mitochondrion and inject a multitude of mitochondrial genomes, this would be helpful, but if only a few, distinct mitochondria are targeted, this might not be sufficient. Even with genetic modifications, xenophagy or dysfunction and lysis of the vector would likely occur at some point, so it does not have an indefinite amount of time.

Instead of bacteria, an intracellular yeast like Cryptococcus neoformans could be used as the mtDNA delivery vector. This may be possible because the yeast Saccharomyces cerevisiae can maintain a mammalian mitochondrial genome in its mitochondria [21]. C. neoformans cells have around 35 mitochondria [22]. Additionally, each yeast mitochondrion may generally contain multiple mitochondrial genomes that are compacted in nucleoid structures, as with mammalian mitochondria. To reduce its size and probably increase its invasiveness, an acapsular version can be utilized. Typically, C. neoformans is 4-6 μm in diameter. Smaller cells may be required to invade certain adult stem and post-mitotic cell types. Fortunately, C. neoformans cells as small as 1.1 μm in diameter have been found [23]. It may take more research to figure out how to limit the size of C. neoformans. Of course, the maximum size of the yeast that can be utilized would be important to transfer the maximum number of mitochondria. Perhaps a diameter of around 2.5 μm would be ideal.

If the yeast are maintained at a low copy number, wherein yeast vacuole fusion is prevented, they could keep pace with the host cell TRM division if that occurs [24]. They could also be secreted

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continuously at low level via expulsion or vacuole lysis and secretory autophagy. It would be retargeted to a cell surface protein that is ubiquitously expressed by adherent cells. Expression of an invasin and listerolysin O could allow it to invade a wide variety of cell types [25,26]. The yeast could enter target cells, lyse their vacuoles, and then lyse to release their mitochondria and replicating RNA encoding TFAM and nucleases. Xenophagy of the yeast mitochondria in target cells could be avoided via the the inclusion of deubiquitylases in the mitochondrial outer membrane. Human mitofusins could be expressed to promote fusion with the target cell mitochondria.

Conclusions

At some point, mitochondrial DNA mutations will need to be addressed. Discussed in this paper is a method of replacing somatically mutated mtDNA throughout the body. While fusion of yeast mitochondria with human mitochondria may not ensue naturally, synthetic biology should be able to rectify that situation if necessary. Interestingly, mouse mitochondria can fuse with human mitochondria [27]. Also, an artificial vector called MITO-Porter can fuse with human mitochondria [28]. There are immunogenicity concerns that will need to be addressed, but this is likely not an insurmountable issue.

References

1. Renteln M. Toward Systemic Lipofuscin Removal. Rejuvenation Research 2024;27(5):171–179; doi: 10.1089/rej.2024.0034.
2. Hayal TB, Wu C, Abraham D, et al. The Impact of CD45-Antibody-Drug Conjugate Conditioning on Clonal Dynamics and Immune Tolerance Post HSPC Transplantation in Rhesus Macaques. Blood 2023;142:3419; doi: 10.1182/blood-2023-182066.
3. Best BP. Nuclear DNA Damage as a Direct Cause of Aging. Rejuvenation Research 2009;12(3):199–208; doi: 10.1089/rej.2009.0847.
4. Vijg J, Schumacher B, Abakir A, et al. Mitigating age-related somatic mutation burden. Trends in Molecular Medicine 2023;29(7):530–540; doi: 10.1016/j.molmed.2023.04.002.
5. Cortese FAB, Santostasi G. Whole-Body Induced Cell Turnover: A Proposed Intervention for Age-Related Damage and Associated Pathology. Rejuvenation Res 2016;19(4):322–336; doi: 10.1089/rej.2015.1763.
6. Bujarrabal-Dueso A, Sendtner G, Meyer DH, et al. The DREAM complex functions as conserved master regulator of somatic DNA-repair capacities. Nat Struct Mol Biol 2023;30(4):475–488; doi: 10.1038/s41594-023-00942-8.
7. Phinney DG, Di Giuseppe M, Njah J, et al. Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. Nat Commun 2015;6:8472; doi: 10.1038/ncomms9472.
8. Wang Q, Yu J, Kadungure T, et al. ARMMs as a versatile platform for intracellular delivery of macromolecules. Nat Commun 2018;9(1):960; doi: 10.1038/s41467-018-03390-x.
9. Mc Cafferty S, De Temmerman J, Kitada T, et al. In Vivo Validation of a Reversible Small Molecule-Based Switch for Synthetic Self-Amplifying mRNA Regulation. Molecular Therapy 2021;29(3):1164–1173; doi: 10.1016/j.ymthe.2020.11.010.
10. Perkovic M, Gawletta S, Hempel T, et al. A trans-amplifying RNA simplified to essential elements is highly replicative and robustly immunogenic in mice. Mol Ther 2023;31(6):1636–1646; doi: 10.1016/j.ymthe.2023.01.019.
11. Ekstrand MI, Falkenberg M, Rantanen A, et al. Mitochondrial transcription factor A regulates mtDNA copy number in mammals. Hum Mol Genet 2004;13(9):935–944; doi: 10.1093/hmg/ddh109.
12. Murakami H, Ota A, Simojo H, et al. Polymorphisms in Control Region of mtDNA Relates to Individual Differences in Endurance Capacity or Trainability. The Japanese Journal of Physiology 2002;52(3):247–256; doi: 10.2170/jjphysiol.52.247.
13. Castañeda V, Haro-Vinueza A, Salinas I, et al. The MitoAging Project: Single nucleotide polymorphisms (SNPs) in mitochondrial genes and their association to longevity. Mitochondrion 2022;66:13–26; doi: 10.1016/j.mito.2022.06.008.
14. Auber M, Svenningsen P. An estimate of extracellular vesicle secretion rates of human blood cells. Journal of Extracellular Biology 2022;1(6):e46; doi: 10.1002/jex2.46.
15. Zekonyte U, Bacman SR, Smith J, et al. Mitochondrial targeted meganuclease as a platform to eliminate mutant mtDNA in vivo. Nat Commun 2021;12(1):3210; doi: 10.1038/s41467-021-23561-7.
16. Shoop WK, Lape J, Trum M, et al. Efficient elimination of MELAS-associated m.3243G mutant mitochondrial DNA by an engineered mitoARCUS nuclease. Nat Metab 2023;5(12):2169–2183; doi: 10.1038/s42255-023-00932-6.

17. Javanpour AA, Liu CC. Genetic compatibility and extensibility of orthogonal replication. ACS Synth Biol 2019;8(6):1249–1256; doi: 10.1021/acssynbio.9b00122.
18. Zhu J, Batra H, Ananthaswamy N, et al. Design of bacteriophage T4-based artificial viral vectors for human genome remodeling. Nat Commun 2023;14(1):2928; doi: 10.1038/s41467-023-38364-1.
19. Yoon YG, Koob MD. Transformation of isolated mammalian mitochondria by bacterial conjugation. Nucleic Acids Research 2005;33(16):e139; doi: 10.1093/nar/gni140.
20. Xu Z, Rao Y, Peng B. Protocol for microglia replacement by peripheral blood (Mr PB). STAR Protocols 2021;2(2):100613; doi: 10.1016/j.xpro.2021.100613.
21. Yoon YG, Koob MD. Intramitochondrial transfer and engineering of mammalian mitochondrial genomes in yeast. Mitochondrion 2019;46:15–21; doi: 10.1016/j.mito.2019.03.006.
22. https://bionumbers.hms.harvard.edu/bionumber.aspx?s=n&v=2&id=108845
23. Feldmesser M, Kress Y, Casadevall A. Dynamic changes in the morphology of Cryptococcus neoformans during murine pulmonary infection. Microbiology 2001;147(8):2355–2365; doi: 10.1099/00221287-147-8-2355.
24. Luo Y, Alvarez M, Xia L, et al. The Outcome of Phagocytic Cell Division with Infectious Cargo Depends on Single Phagosome Formation. PLoS ONE 2008;3(9):e3219; doi: 10.1371/journal.pone.0003219.
25. Grillot-Courvalin C, Goussard S, Huetz F, et al. Functional gene transfer from intracellular bacteria to mammalian cells. Nat Biotechnol 1998;16(9):862–866; doi: 10.1038/nbt0998-862.
26. Zare M, Farhadi A, Zare F, et al. Genetically engineered E. coli invade epithelial cells and transfer their genetic cargo into the cells: an approach to a gene delivery system. Biotechnol Lett 2023;45(7):861–871; doi: 10.1007/s10529-023-03387-7.
27. Yoon YG, Haug CL, Koob MD. Interspecies mitochondrial fusion between mouse and human mitochondria is rapid and efficient. Mitochondrion 2007;7(3):223–229; doi: 10.1016/j.mito.2006.11.022.
28. Yamada Y, Fukuda Y, Harashima H. An analysis of membrane fusion between mitochondrial double membranes and MITO-Porter, mitochondrial fusogenic vesicles. Mitochondrion 2015;24:50–55; doi: 10.1016/j.mito.2015.07.003.