

Mitochondrial DNA and Ageing

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Abstract

Mitochondrial DNA is a separate genome located in the cytoplasm of nearly all eukaryotic cells. Its importance in developmental outcome has often been neglected. However, its transmission and replication are strictly regulated during early development, as they are integral to the viability and health of the offspring. The accumulation of mitochondrial DNA mutations has been proposed as a potential mechanism in the physiological process of ageing and age-related disease. Although mitochondria have long been anticipated as a perpetrator of ageing, there was little experimental evidence to link these changes directly with the cellular pathology of ageing. Recently, considerable progress in understanding basic mitochondrial genetics and in identifying acquired mtDNA mutations in ageing has been made. Furthermore, the creation of mtDNA-mutator mice has provided the first direct evidence that accelerating the mtDNA mutation can result in premature ageing, consistent with the view that loss of mitochondrial function is a major causal factor in ageing. This review will, therefore, focus on recent developments in ageing research related to the role played by mitochondrial DNA.

Keywords: Mitochondria, DNA, mitochondrial diseases, ageing, inflammation, mutations, deletions, ROS production.

Introduction

Mitochondria plays a central role in ageing ¹. The initial free radical theory suggested that reactive oxygen species (ROS), which are constantly generated through normal metabolism, are causing ageing, as well as associated degenerative diseases, by introducing irreversible damage to membranes, proteins and DNA ². Early studies on isolated mitochondria, where many of the redox elements were held in an artificial state, have suggested that 1–4% of the total oxygen consumed was used to generate ROS ³. However, subsequent studies,

under more physiological conditions, have estimated this basal value to ~0.2% ⁴. Even with this lower estimated value of ROS generation, the mitochondrial electron transport chain (ETC) still remains the major site of ROS production in a cell. This fact has led to the suggestion that mitochondria are also prime targets for oxidative damage and that accumulation of defective mitochondria is a major contributor to ageing⁵. Indeed, an increased number of dysfunctional mitochondria are found in a number of animal models and human tissues with increasing age ⁶. Besides, mitochondria are the only organelles in the animal cell that possess their own DNA, mitochondrial DNA (mtDNA), with the limited arsenal of DNA repair processes, further supporting the idea that mitochondria might be particularly sensitive to accumulation of damage⁷. The mitochondrial theory of ageing, a correlate of the free radical theory, is also based around the idea of a vicious cycle, in which somatic mutations of mitochondrial DNA provoke respiratory chain dysfunction leading to enhanced ROS production and in turn to the accumulation of further

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mtDNA mutations⁸. Harman's conjecture was that the generation of reactive oxygen species (ROS), which he viewed as likely coming from the mitochondria, gave rise to the subsequent accumulation of damaged proteins, lipids, and DNA, thereby fueling aging and age-related diseases in an inevitable but stochastic process⁹. While the notion that increased mitochondrial ROS directly causes aging has fallen into disfavor, in its wake, other aspects of mitochondrial biology have grown increasingly more appealing¹⁰. Some links between aspects of mitochondrial biology and aging have been the subject of many excellent recent reviews¹¹. Here, we focus on those aspects of mitochondrial physiology that have perhaps the strongest connection to human aging. These processes include the emerging role that mitochondria play in inflammation¹²; how dysregulation of mitochondrial quality control and age-related mitochondrial dysfunction contributes to aging, age-related mitochondrial dysfunction, and the notion of retrograde signaling; and why a little mitochondrial stress might ultimately be a good thing¹³.

mt DNA:

Mitochondria are structures within cells that convert the energy from food into a form that cells can use¹⁴. Each cell contains hundreds to thousands of mitochondria, which are located in the fluid that surrounds the nucleus (the cytoplasm). Although most DNA is packaged in chromosomes within the nucleus, mitochondria also have a small amount of their own DNA. This genetic material is known as mitochondrial DNA or mtDNA¹⁵. In humans, mitochondrial DNA spans about 16,500 DNA building blocks (base pairs), representing a small fraction of the total DNA in cells¹⁶. Mitochondrial DNA contains 37 genes, all of which are essential for normal mitochondrial function. Thirteen of these genes provide instructions for making enzymes involved in oxidative phosphorylation¹⁷. Oxidative phosphorylation is a process that uses oxygen and simple sugars to create adenosine triphosphate (ATP), the cell's main energy source. The remaining genes provide instructions for making molecules called transfer RNA (tRNA) and ribosomal RNA (rRNA), which are chemical cousins of DNA. These types of RNA help assemble protein building blocks into functioning proteins¹⁸. mtDNA is a separate genome located in the cytoplasm of nearly all eukaryotic cells. Its importance in developmental

outcome has often been neglected. However, its transmission and replication are strictly regulated during early development, as they are integral to the viability and health of the offspring¹⁹. Failure to regulate these processes during oogenesis, in the embryo, and prior to gastrulation can have deleterious consequences for the fetus and the offspring. Human mitochondrial DNA was the first significant part of the human genome to be sequenced. This sequencing revealed that the human mtDNA includes 16,569 base pairs and encodes 13 proteins. Since animal mtDNA evolves faster than nuclear genetic markers it represents a mainstay of phylogenetics and evolution. It also permits an examination of the relatedness of populations, and so has become important in anthropology and biogeography.

Mitochondrial DNA and disease:

Most mtDNA alterations are neutral polymorphisms, which have proved useful in tracking human migrations²⁰. The first pathogenic mtDNA mutations were identified in 1988²¹. Since then, over 250 pathogenic mtDNA mutations (point mutations and rearrangements) have been characterised, shown to cause a wide variety of diseases with a heterogeneity of phenotypes and a variable age of onset²². The exact prevalence of mtDNA disease is notoriously difficult to ascertain due to clinical heterogeneity of mitochondrial diseases and the plethora of known causative mutations. The onset of clinical symptoms, phenotypic variability, and variable penetrance of mitochondrial diseases are governed by a number of factors, including the threshold effect, mitotic segregation, clonal expansion, and a genetic bottleneck. Leigh syndrome is a progressive neurodegenerative condition, which particularly affects the brain, diencephalon, and basal ganglia. There are characteristic neuropathological features, but newer neuroimaging techniques can now easily detect these lesions in life. Leigh syndrome is due to severe failure of oxidative metabolism and can be due to a variety of different genetic defects affecting the mitochondrial genome. The clinical features associated with depletion syndromes depend upon the organ(s) which have tDNA depletion⁷. On the whole, these are severe disorders and present in childhood with severe muscle weakness, progressive encephalopathy, or liver failure. Pearson syndrome is a rare disorder of infancy characterised by sideroblastic anaemia with pancytopenia and exocrine

pancreatic failure²³. The clinical course in these children can be severe leading to early death. In those that survive the blood disorder improves but they later develop the clinical features of KSS. In these children, there is a very high level of large-scale single mtDNA deletion present in all tissues²⁴. One of the most common presentations of mtDNA disease in adults is CPEO. CPEO is characterised by a progressive paralysis of the eye muscles leading to impaired eye movement and ptosis.

Mitochondria and the inflammation of ageing:

Ageing is associated with evidence of an activation of the innate immune system that leads to a condition known as “inflammaging”²⁵. This is particularly true of elderly individuals who age less successfully than their peers, as both the frail and the pre-frail exhibit higher circulating levels of inflammatory mediators such as C-reactive protein, IL-6, and fibrinogen²⁶. These relationships exist even in the absence of associated age-related diseases (e.g., cancer, cardiovascular disease, etc.), suggesting a potential causative relationship between activation of the immune system and accelerated aging. Perhaps because of its stability and ease of measurement, the data are most compelling with respect to circulating IL-6, as elevated IL-6 serum levels are predictive of incident disability, frailty, walking speed, and overall mortality. Analysis of T cells from elderly individuals suggests that although basal levels of cytokine production are elevated, invoked responses to a cytokine challenge are reduced²⁷. Besides evidence for augmented basal cytokine signaling, chronic activation of the inflammasome with elevated IL-1 β levels appears to identify a subset of elderly individuals with higher rates of essential hypertension, arterial stiffness, and all-cause mortality. Consistent with this, evidence for activation of the NLRP3 inflammasome is seen in a wide range of age-related conditions, including atherosclerosis, Alzheimer’s disease and metabolic syndrome. Interestingly, a number of preclinical observations suggest that the activation of the inflammasome may be maladaptive²⁸. There is increasing evidence that mitochondria might play an important role in the inflammaging phenotype²⁹. The immune system is capable of sensing and responding to tissue damage and views the release of intracellular molecules in an analogous fashion to dangerous pathogens. This “danger theory” argues that certain molecules released from senescent or dying cells might constitute signals

that trigger an immune response, often termed damage-associated molecular patterns (DAMPs). In that context, the mitochondria, derived several billions of years ago from the endosymbiosis of α -proteobacteria into a eukaryotic cell, represent a smorgasbord of potential immune-stimulating DAMPs. Putative mitochondrial DAMPs include the release of mitochondrial DNA (mtDNA), *N*-formyl peptides generated by translation of mitochondrial-encoded protein, and unique lipid species such as cardiolipin, a unique phospholipid enriched on the inner mitochondrial membrane. In the case of circulating mtDNA, immune activation occurs, in part, by activation of Toll-like receptor 9 (TLR9) signaling, while for mitochondrial proteins, activation occurs through binding to formyl peptide receptor-1. This was first demonstrated in the case of trauma, where the release of mtDNA and formyl peptides activated circulating neutrophils to mediate tissue injury.

Functional significance of mtDNA:

Mitochondrial DNA has strands. These strands are present in the ratio of 1 mitochondrion : 2- 10 Mitochondrial DNA(mtDNA). Humans inherit them maternally. The involved mechanisms include dilution of sperm mtDNA within the fertilized egg. The uniparental mtDNA pattern is found in most animals and plants. The strands are susceptible to oxygen - reactive species and contain proteins with significant DNA repair capacity. They are susceptible to oxidative damage. The mtDNA mutations cause inherited diseases and aid the process of ageing. There are anything between 100 and 10,000 copies of mtDNA present per human cell. This genetic material is rearranged via recombination and remains unchanged, when passed on from parent to offspring. The mtDNA helps track ancestry back to hundreds of generations. It is compared in forensic science, to identify corpses and skeletal remains that are unidentified. The strands are used in combination with anthropological and missing persons. mtDNA facilitates the assessment of genetic relationships between individuals and quantifying evolutionary relationships. Dedicated mtDNA data helps biologists to construct a relationship network among various sequences. The sequences become larger, with evidence of a distant or non - existent relationship between species. It is highly susceptible to somatic or non - inherited mutation³⁰. These occur within the DNA of some cells and are not passed

down generations. Classic somatic mutations in mtDNA are seen in cancer of the colon, breast, liver and stomach and even leukemia and lymphoma. Mitochondrial DNA is not able to completely repair itself and hence, this leads to additional somatic mutations. When building blocks of nucleotides are altered, the energy producing ability of mitochondria is reduced and this leaves the immune system fighting infection with little or no help from these strands³¹. The DNA strand is very important to biologists and forensic experts when attempting to establish vital relationships between people. It is being explored to answer the common questions that have plagued man for centuries on the possible evolutionary routes and possible age-reversing. Within the human body, these essential building blocks back up and strengthen the immune system to keep a number of ailments at bay³².

Mitochondrial DNA keeps us young:

The extended inflammatory infiltrates suggest that mitochondria could produce ROS can act as signaling molecules for inflammasome activation³³; unfortunately, there are no report measurements of oxidative stress, but cells depleted of mtDNA are usually characterized by diminished oxygen consumption and ROS production³⁴, suggesting that oxidative stress should not mediate the ageing phenotype observed here. However, the following two major consequences were observed in a cell model of mtDNA depletion using the same strategy as that used in the depletor mouse; a significant rearrangement of histone acetylation due to indirect alterations in the citrate levels and a reduction in cell proliferation due to a reduction in the membrane potential and destabilization of Hif1 α . While the type of epigenetic rearrangement that occurs during ageing is unclear. Hif1 α depletion has been shown to lead to an accelerated aged skin phenotype in mice. Additionally, skin mitochondria with depleted DNA display a loss of mitochondrial cristae presumably due to the loss of F1/FO ATP synthase dimers. Indeed, the rearrangement of ATP synthase dimers could predispose cells to death by opening the permeability transition pore channel³⁵. There is no report of measure of cell death (that was observed in the mutator mouse); nonetheless, since multiple mitochondrial molecules can act as damage-associated molecular patterns (DAMPs), an increase in PTPC-mediated cell death could cause the extensive

release of mito-DAMP and, ultimately, the observed increase in inflammation. The recovery of the skin phenotype is more intriguing. The mutator mouse model provided important insight into how mitochondria can induce an ageing phenotype by affecting haematopoietic and neural stem cell self-renewal capacities³⁶.

Conclusion

There is a growing body of evidence that mitochondria play a central role in the ageing process. Mitochondrial dysfunction and mtDNA mutations have been shown to amplify during the course of ageing. Results obtained from mtDNA mutator mice have further strengthened the role of mitochondria in organismal ageing. However, lack of increased oxidative stress in the mtDNA mutator mice has raised reasonable doubts in the direct connection of mtDNA mutations with increased ROS production. However, this does not imply that there is no age-dependent accumulation of oxidative stress and increased ROS production, neither is it a final proof that oxidative stress does not play an important role in the generation of mtDNA mutations. Indeed, there is extensive literature demonstrating amplified oxidative damage to the different cell compartments with increasing age, not least in mitochondria and mtDNA. However, reviewing this literature is beyond the scope of this article that was intended to focus primarily on the role of mtDNA mutations in ageing. The role of mitochondrial dysfunction in human ageing must now be revisited and additional studies of respiratory chain capacity and mtDNA mutation load in different organs from humans of different ages must be performed to further validate this hypothesis. It may also be possible to perform additional genetic experiments in the mouse to test this hypothesis, for example creating mice with enhanced mtDNA polymerase proofreading capacity or enhanced mtDNA repair capacity. Such mouse mutants should have a longer than normal life span if somatic mtDNA mutations have a causative role in ageing. Mitochondria are now at the center stage in human disease and ageing and we should look forward to exciting developments in this field during the coming years.

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