Somatic mtDNA mutations and aging – Facts and fancies

Alexandra Kukat, Aleksandra Trifunovic *

Division of Metabolic Diseases, Department of Laboratory Medicine, Karolinska Institute, S-14186 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 5 May 2008
Accepted 14 May 2008
Available online 21 May 2008

Keywords:
mtdNA mutator mice
Aging
mtDNA deletion
mtDNA point mutation

ABSTRACT

Mitochondria play a critical role in the life of the cell as they control their metabolic rate, energy production and cell death. Mitochondria have long been appreciated as causative to aging. The age-associated respiratory chain deficiency is typically unevenly distributed and affects only a subset of cells in various human tissues, such as heart, skeletal muscle, colonic crypts and neurons. Studies of mtDNA mutator mice has provided the first direct evidence that accelerating the mtDNA mutation rate can result in premature aging, consistent with the view that loss of mitochondrial function is a major causal factor in aging. New, controversial data have arisen from the studies on molecular mechanisms that drive premature aging in mtDNA mutator mice. Our results suggest that the accumulation of high levels of mtDNA point mutations, causing amino acid substitutions, combined with their clonal expansion is probably the main driving force behind premature aging in mtDNA mutator mice.

© 2008 Elsevier Inc. All rights reserved.

1. Mitochondrial DNA mutations as a driving force in the aging process

The involvement of mitochondrial DNA (mtDNA) in the aging process mainly stems from their location, in close proximity to the mitochondrial respiratory chain, which is the main source of reactive oxygen species (ROS) generation and therefore helps explain their susceptibility to oxidative damage. This vulnerability of mtDNA has led to the suggestion that the accumulation of somatic mtDNA mutations might play an important role in the aging process by producing cells with a decreased oxidative capacity (Harman, 1972).

The damage to mtDNA is associated with deleterious functional alterations in the activity of electron transport chain (ETC) complexes since they are partly encoded by mtDNA. The number of cytochrome c oxidase (COX – complex IV of ETC) deficient cells progressively increases in postmitotic tissues (skeletal muscle, cardiomyocytes and brain) of elderly humans (Cottrell et al., 2001; Mueller-Hocker, 1989; Müller-Höcker, 1990). Furthermore, an age associated increase in the number of COX-deficient cells is also found in highly proliferative tissues like the stem cells of colonic crypts (Taylor et al., 2003).

A general decline in the oxidative phosphorylation capacity has been reported in aging human tissues including skeletal muscle, liver, heart and brain (Cottrell and Turnbull, 2000). It has been proposed that this could be the result of an imbalance in the activity ratios among different complexes that may affect oxidative phosphorylation and lead to an elevated ROS production (Kwong and Sohal, 2000).

2. Mitochondrial DNA deletions in aging

Rearrangements and deletions of the mitochondrial genome have been correlated with aging in a variety of species as diverse as Caenorhabditis elegans (Melov et al., 1995), Drosophila melanogaster (Yui et al., 2003), mice (Piko et al., 1988), rats (Gadaleta et al., 1992) dogs (Sugiyama et al., 1993) and primates (Lee et al., 1993). Most mtDNA deletions detected in aging tissues share similarities with the ones detected in patients suffering from mitochondrial diseases, e.g., progressive external ophthalmoplegia (PEO). Polymerase chain reaction (PCR) analysis allows the identification of small populations of deleted mtDNA that are undetectable by Southern blot hybridization, a technique commonly used to detect large-scale mtDNA deletions (Krishnan et al., 2008). The majority of mtDNA deletions is located in the major arc between the two proposed origins of replication (O4 and O2; Mitomap), and is predominantly (85%) flanked by short direct repeats. However, the levels of deleted mtDNA molecules associated with aging are too low to be discernable by Southern blot hybridization, a technique commonly used to detect high amounts of mtDNA deletions in patients suffering from mitochondrial diseases, e.g., progressive external ophthalmoplegia (PEO). Polymerase chain reaction (PCR) analysis allows the identification of small populations of deleted mtDNA that are undetectable with Southern blot. Further refinements of PCR techniques enable reliable quantification of the fraction of deleted mtDNA molecules. These quantitative estimates are based on comparisons of the intensities of ethidium bromide-stained or radiolabelled PCR products relative to those obtained after dilutions of PCR fragments, that were either derived from a different region of the mitochondrial genome or from the region of wild-type mtDNA where the deletion occurred.

* Corresponding author. Tel.: +46 8 5858 3677; fax: +46 8 779 5383.
E-mail address: alexandra.trifunovic@ki.se (A. Trifunovic).

0531-5565/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.exger.2008.05.006
The most frequently observed deletion in humans is the “common deletion” that spans 4977 nucleotides and occurs at a presumed deletion “hot spot” involving two 13 bp direct repeats (Holt et al., 1988). The “common deletion” is often accompanied by mtDNA7436 and mtDNA10422 deletions. Quantitative analysis of different tissues of elderly humans confirmed the accumulation of all detected mtDNA deletions, but the detected levels vary considerably. One of the earliest studies showed that the amount of the “common deletion” in the heart and brain of older individuals was around 0.1% but was undetectable in fetal tissues (Cortopassi and Arnheim, 1990). Another study reported the appearance of mtDNA deletions in the heart beginning at 40 years of age with subsequent accumulation with increasing age. In this study, the “common deletion” was accompanied by the mtDNA7436 and mtDNA10422 deletions and reached a maximum of 0.007% (Corral-Debrinski et al., 1992b). However, much higher levels of the mtDNA7436 deletion in human hearts were detected in 80- and 90-year-old individuals (3% and 9%, respectively) (Sugiyama et al., 1991). Detection and quantification of the “common deletion” and the mtDNA7436 deletion in the cortex, putamen, and cerebellum showed a significant increase in elderly humans reaching a maximum of deleted mtDNA molecules of 12% in the putamen, 3.4 % in the temporal cortex but only 0.1% in the cerebellum (Corral-Debrinski et al., 1992a). Similar results were obtained from a detailed study set to determine the levels of the “common deletion” in 12 different brain regions. The highest level of accumulation (up to 46% of deleted molecules) was detected in the caudate, putamen and substantia nigra of an 82-year-old individual (Soong et al., 1992). These regions are characterized by high dopamine metabolism, which leads to an increased H2O2 production by the breakdown of dopamine by monoamine oxidase B. The lowest level was detected in the white matter and cerebellum probably reflecting differences in the local metabolic rate of glucose utilization and therefore different rates of ROS formation (Soong et al., 1992). An extensive study including heart, skeletal muscle, brain, diaphragm muscle, kidney, spleen, skin and liver revealed the occurrence of the “common deletion” in all examined tissues from older individuals. Higher levels of deletions were detected in postmitotic tissues i.e., skeletal muscle, heart and brain in contrast to kidney, spleen, skin and liver that are composed of dividing cells, indicating a tissue-specific “pattern” of deletion accumulation (Cortopassi et al., 1992).

All studies previously mentioned were specifically designed to detect a single type of mtDNA deletion. With the use of long-extension PCR (LX-PCR) that can amplify the entire mitochondrial genome it is possible to detect the whole spectrum of mtDNA deletions. While the LX-PCR procedure is not quantitative, it reveals a dramatic qualitative difference between the mtDNA of young and old tissues. Multiple mtDNA mutations progressively accumulate with age in skeletal muscle to the extent that little or no full-length mtDNA is present in old aged subjects (Kovalenko et al., 1997). Furthermore, the mtDNA rearrangements appear to be highly heterogeneous and differ not only between tissues but also between different regions within one tissue (Kovalenko et al., 1997).

Further refinements were made by the analysis of single cells taking into account that mtDNA mutations may not be distributed equally in a given tissue but might show a focal accumulation due to clonal expansion of mutations in single cells. Cells that accumulate a large number of mitochondrial mutations and have reduced levels of full-length mtDNA would be expected to be severely affected and show reduced COX activity as a consequence. While single COX positive skeletal muscle fibers of young and aged subjects showed mainly full-length mtDNA and few, if any, mtDNA rearrangements, COX deficient fibers taken from the same individuals, depicted a heterogeneous population of rearranged mtDNA molecules, often with no detectable full-length mtDNA (Kopsidas et al., 1998). These results demonstrate that COX deficient fibers in young and old individuals have the same mtDNA rearrangements but the number of COX-negative cells is increasing with age (Kopsidas et al., 1998). Analysis of single cardiomyocytes isolated from the heart of centenarians revealed that one out of seven analyzed cells carried a significant proportion of different deletions ranging from 2% to 64% (Khrapko et al., 1999). Furthermore, each of those deletion-rich cells contained only one type of deletion, most likely derived from a single initial mutational event and clonal expansion (Khrapko et al., 1999). Similar results, with clonally expanded mtDNA deletions of more than 60%, were obtained for individual substantia nigra neurons of aged individuals (Kraytsberg et al., 2006). It has been proposed that deleted mtDNA molecules, being smaller, could possess a replicative advantage that is most efficient in the situation of relaxed copy number control as it takes place in postmitotic tissues (Diaz et al., 2002).

3. Mitochondrial point mutations and aging

Age-associated accumulation of point mutations in human mtDNA had been more difficult to assess mainly for technical reasons. Therefore, first analyses were focused on looking for the mutations already known to occur in different mitochondrial diseases. The very first report taking point mutations into account as a causative factor in aging was made by Münscher et al. by amplifying only mutated DNA with primers containing a mismatched 3’ residue. A small amount of the A8344G transition, a mtDNA mutation commonly associated with Myoclonus Epilepsy Associated with Ragged Red Fibers syndrome (MERRF), was found in the extracellular muscle of individuals older than 74 years of age (Münscher et al., 1993). The amount of mutated mtDNA was estimated to be 2.0–2.4%, and was often associated with the presence of the “common deletion” (Münscher et al., 1993). In a similar study, elevated levels of the A3243G mutation, a mutation typical for mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke (MELAS), were found mainly in heart, brain and skeletal muscle of aged individuals (Zhang et al., 1993). Using optimized, more sensitive PCR conditions, it was shown that this mutation could be detected in all tissues (skeletal muscle, brain, heart, liver, kidney) from both infants and adults, but the amount was 10-fold higher in the adult tissues (0.1%) (Liu et al., 1997). These data suggest that the accumulation of mtDNA mutations starts very early in life, probably due to high mtDNA replication during embryogenesis. In addition, elevated levels of the “common deletion” were found in the same individuals. Interestingly, the mean abundance of the “common deletion” is substantially higher in skeletal muscle than in heart and kidney in contrast to the amount of the A3243G mutation that was found to be lower in skeletal muscle than in heart and kidney (Liu et al., 1997, 1998). A detailed study was proposed to examine different mtDNA mutations in skeletal muscle samples of human subjects from just after birth (i.e., 1 h) to 90 years of age. Base substitutions showed in general a very early onset since three mutations were detected in the muscles of infants aged both 1 h and 5 weeks of age (Zhang et al., 1998). However, only one mutation (A3243G) showed significant accumulation with age, while the others occurred only sporadically without age-dependent accumulation. Actually, one of the detected mutations (A13167G) showed a significant decline in its abundance as a function of age (Zhang et al., 1998). Furthermore, the specific mutations were found to occur independent of each other, indicating the random nature of the mtDNA point mutations. Another study, using a different quantification method, found no evidence of an age-dependent accumulation of either A3243G or A8344G mutations in skeletal muscle and brain of aged individuals (Murdock et al., 2000).
Point mutations associated with the major regulatory region for replication were studied by an approach based on the use of purified mtDNA, enzymatic digestion and denaturing gradient gel electrophoresis (DGGE) that can identify single nucleotide mismatch in an artificially produced heteroduplex (Michikawa et al., 1999). The T414G transversion that lies in the middle of the promoter for primer synthesis for H-strand replication and L-strand transcription was found in a generally high proportion (up to 50%) in the skin fibroblasts of individuals above 65 years of age (Michikawa et al., 1999). This mutation could not be detected in skeletal muscle samples, although two new point mutations (A189G and T408A) at the mtDNA replication control sites, were accumulated in age-dependent and tissue specific manner in the muscles of old individuals (Wang et al., 2001).

Similar to age-associated accumulation of mtDNA deletions, a real breakthrough occurred when the search for point mutations was conducted in a single cell. MtDNA sequence analyses in single cells have shown that a number of cells in diverse tissues contain a high proportion of clonally expanded mutant mtDNA molecules. Analysis of five tRNA genes showed high levels of clonally expanded mtDNA point mutations (49–94%) in COX-deficient muscle fibers isolated from aged individuals (Fayet et al., 2002). In another study, a different mutational spectrum has been found in a dividing tissue (epithelial cells) compared to a postmitotic tissue (cardiomyocytes) from a single individual (Nekhaeva et al., 2002). A recent study showed that the mean number of mutations per mitochondrial genome in single neurons and glial cells of older individuals was 3.3% and 2.2%, respectively. Additionally, each cell contained multiple distinct somatic mutations most of which were present at a very low level. These results indicate that even if the total level of any particular mtDNA mutation might be low, the load of all present mutations in a cell can be substantial and lead to mitochondrial dysfunction (Cantuti-Castelvetri et al., 2005). The first study describing an age-associated accumulation of point mutations in human stem cells of colonic crypts reported numerous point mutations in both heteroplasmic and homoplasmic states. These mutations caused a severe COX-deficiency that is passed from the stem cells to their progeny (Taylor et al., 2003).

Computer simulations based on experimentally derived biological parameters that reflect understanding of the mtDNA replication were carried out, indicating that random genetic drift alone might lead to the clonal accumulation of mutant mtDNA within single postmitotic cell (Elson et al., 2001). Thus, somatic mtDNA mutations occurring during a critical period throughout childhood and early adulthood have sufficient time to reach significant levels by random genetic drift alone (Elson et al., 2001). These observations challenge the vicious cycle hypothesis, which proposes an accumulation of mtDNA mutations due to an age-related decline in respiratory chain efficiency and increased ROS production.

4. mtDNA mutator mice

Recently, we created homozygous knock-in mice expressing a proofreading deficient form of the nuclear-encoded mitochondrial DNA polymerase (Polγ) (Trifunovic et al., 2004). The introduced mutation was designed to create a defect in the proofreading function of Polγ, leading to the progressive, random accumulation of mtDNA mutations during the course of mitochondrial biogenesis. As the proofreading in the knock-in mice is efficiently prevented, these mice develop an mtDNA mutator phenotype (mtDNA mutator mice) with a three to fivefold increase in the levels of point mutations. Surprisingly, increased levels of mtDNA mutations were not associated with increased ROS production or increased oxidative stress in mtDNA mutator mice (Trifunovic et al., 2005). The mtDNA mutator mice display a completely normal phenotype at birth and in early adolescence but subsequently acquire many features of premature aging such as weight loss, reduced subcutaneous fat, alopecia, kyphosis, osteoporosis, anemia with progressive decrease in circulating red blood cells, reduced fertility, cardiomyopathy and sarcopenia (Trifunovic et al., 2004). Confirmation of our results came from another, independently developed mtDNA mutator mice (Kujoth et al., 2005). High levels of apoptotic cells were detected in both proliferating (thymus, intestine, testis) and postmitotic tissues (skeletal muscle, brain) in both mtDNA mutator strains (Kujoth et al., 2005; Niu et al., 2007).

A progressive and random accumulation of mtDNA point mutations was rather uniform in different tissues, suggesting that much of the mutation accumulation may have occurred during embryonic and/or fetal development (Trifunovic et al., 2004). Indeed, the mutation load was already substantial in mtDNA mutator at embryonic day 13.5 (7.8 ± 0.4 mut/10 kb compared to 1.5 ± 0.9 mut/10 kb in wild-type embryos) (Trifunovic et al., 2005). In addition, mtDNA mutator mice accumulate mtDNA mutations in a linear fashion, suggesting no involvement of a vicious circle predicted by the mitochondrial theory of aging (Trifunovic et al., 2005). However, it is still possible that mutation load might be an underestimate since cells with the highest levels of deleterious mutations may be lost due to cell death and/or replicative disadvantage.

Aside progressive increase in the level of somatic point mutations we have detected the existence of relatively high levels (~25%) of linear mtDNA molecules in different tissues of mtDNA mutator mice. The linear mtDNA molecules span approximately 12 kb between the two origins of mtDNA replication (Ox and O2) (Trifunovic et al., 2004). We believe them to be a consequence of stalled replication due to the loss of Polγ exonuclease activity. Our results strongly argue against their role in the development of the observed phenotypes in mtDNA mutator mice since these linear mtDNA molecules could be detected at very early age (already at embryonic day 13.5), their amount did not change over time, the levels were comparable in all investigated tissues and they are not replicated or transcribed.

MtDNA mutator mice display ~25% decrease in the levels of full-length mtDNA when compared to wild-type mice (Trifunovic et al., 2004). The reduced copy number of full-length mtDNA did not affect overall mtDNA expression (Edgar et al., in preparation). These findings are consistent with our previous observation that ~35–40% reduction of mtDNA copy number in heterozygous germ-line Tjam (mitochondrial transcription factor A) knockout mice has minimal effects on mtDNA expression and respiratory chain function (Larsson et al., 1998). However, we cannot completely exclude a possibility that described reduction in the levels of full-length mtDNA, combined with the high levels of mtDNA mutations could contribute to the phenotypes.

A recent study suggests that circular mtDNA molecules with large deletions and not the above-mentioned abundant mtDNA changes are the driving force behind the premature aging in mtDNA mutator mice (Vermulst et al., 2008). However, we could not detect any deleted, circular molecules after performing LX-PCR analysis of mtDNA with two different primer pairs mapping outside the proposed deleted region (Vermulst et al., 2008) (Edgar et al., in preparation). Using the same method and in the same conditions we were able to detect multiple deletions in mtDNA “deleter” mice. This mouse model overexpresses the mtDNA helicase (Twinkle) carrying a dominant mutation causing adult-onset progressive external ophthalmoplegia (PEO) (Tyynismaa et al., 2005). Overexpression of the mutant Twinkle-PEO protein in mice results in the accumulation of multiple mtDNA deletions (around 5%), progressive respiratory dysfunction and chronic-late-onset mitochondrial dysfunction. The “deleter” mice do not, however, show premature aging phenotypes and have a normal lifespan (Tyynismaa et al., 2005). In another model, mice harboring high propor-
tions of a single, large mtDNA deletion (mito-mice), had a mosaic distribution of COX-deficient cells in both heart and skeletal muscle, and lactic acidosis, that is indicative of mitochondrial dysfunction (Inoue et al., 2000). However, these mice die around 6 months of age due to renal failure and they do not show any signs of premature aging (Inoue et al., 2000).

Large circular mtDNA deletions typically span over several protein coding genes and include a number of tRNA genes. Therefore, consequences of the large deletions could be detected as a reduction in mitochondrial transcript levels or as a decrease in translation of mitochondrially-encoded proteins. We could not detect any decrease in the mitochondrial transcript levels or in the rate of mitochondrial protein synthesis (Edgar et al., in preparation). Instead, the steady-state level of assembled respiratory chain complexes I, III and IV was significantly decreased, probably due to the high mutational load in mitochondria-encoded subunits (Edgar et al., in preparation). Therefore, we believe that even if large mtDNA deletions exist in minute amounts, they could be only a "tip of the iceberg" and not the driving force behind premature aging in mtDNA mutator mice. Our results strongly argue that the observed phenotypes are a direct consequence of the accumulation of mtDNA point mutations. We propose that even though mtDNA mutator mice randomly accumulate point mutations, these mutations would have a deleterious impact primarily on the protein-coding genes. This is in agreement with our recent finding of strong purifying selection against mutations in the protein-coding genes during maternal transmission of mutated mtDNA in the mouse (Stewart et al., 2008).

5. Conclusions

Our results from the mtDNA mutator mice demonstrate that somatic mtDNA mutations have the capacity to cause a variety of aging phenotypes in mammals. However, we do not yet know the relative importance of somatic mtDNA mutations in mammalian aging. One of the reasons is that the overall mutation load found in normal aging tissues is much lower than the one needed to cause mitochondrial dysfunction. Results from the single cells argue that despite a relatively low overall level of mtDNA mutations, the mutational load in single cells could be substantial. Therefore, this may result in a functional impairment of the tissue by the loss of critical cells by cellular senescence or cell death.

We believe that more experiments are needed in order to shed more light on the significance of somatic mtDNA mutations and their role in aging. We are looking forward to gain more insights in this exciting field.

Acknowledgments

A.T. is supported by Grants from the Swedish Research Council, Åke Wiberg Foundation, Funds of Karolinska Institutet and Loo and Hans Östermans Foundation. A.K. is supported by the Grant from The Wenner-Gren Foundation.

References


