Perspective

The Role of Mitochondria in AMD: Current Knowledge and Future Applications

Mohammad Riazi-Esfahani[1,2], MD; Baruch D. Kuppermann[1,3], MD, PhD; M. Cristina Kenney[1,4], MD, PhD

[1]*Department of Ophthalmology, Gavin Herbert Eye Institute, University of California Irvine, Irvine, California, USA*
[2]*Eye Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran*
[3]*Department of Biomedical Engineering, University of California Irvine, Irvine, California, USA*
[4]*Department of Pathology and Laboratory Medicine, University of California Irvine, Irvine, CA, USA*

Abstract

Mitochondria are organelles which comprise the main respiratory machinery in the eukaryotic cells. In addition to their crucial role in energy production, they have profound effects on apoptosis and retrograde signaling to nucleus. Mitochondria have their own DNA, which codes for different proteins mostly involved in oxidative phosphorylation. Significant changes in the mitochondria of retinal pigment epithelium have been reported in age-related macular degeneration (AMD), which is correlated with the severity of the disease. Cybrid cell lines that have identical nuclei but mitochondria from different individuals can provide a unique means for studying the relationship between mitochondria and AMD. Different approaches for protection of mitochondria have been introduced which can be considered as potential future treatments for AMD and other age- related disorders.

Keywords: Age-related Macular Degeneration, DNA, Mitochondria, Mitochondrial

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MITOCHONDRIA AND AGING

It is generally accepted that in eukaryotes the mitochondria comes from an endosymbiotic relationship and its DNA can be linked to an alpha-proteobacterial genome.[1,2] The mitochondria energy requirements determine their count in each cell. Their numbers differ from one to thousands. Each mitochondrion is composed of an intermembrane space surrounded by an outer membrane and an inner membrane, numerous cristae, and the matrix [Figure 1]. Many enzymes are engaged in ATP production. The translocase outer membrane (TOM) and translocase inner membrane (TIM) are the main enzymes for transport of proteins that are encoded by the nuclear DNA (nDNA) into the mitochondria.[3] Interestingly, mitochondria carry their own DNA (mtDNA), which is obtained through maternal lineage. The mtDNA is a closed ring including 16,569 nucleotide pairs and two strands. The heavy strand encodes for 28 genes while the light strand encodes for 9 genes, which yield 13 proteins for oxidative phosphorylation, 2 ribosomal RNAs and 22 transfer RNAs.[4-6] The mtDNAs have multiple copies per cell, unlike nuclear DNA, which has a single copy in each cell.

Mitochondria play an important role in formation of energy, reactive oxygen species (ROS),

Correspondence to:
M. Cristina Kenney, MD. Gavin Herbert Eye Institute, Ophthalmology Research Laboratory, University of California Irvine, California 92697, USA.
E-mail: mkenney@uci.edu

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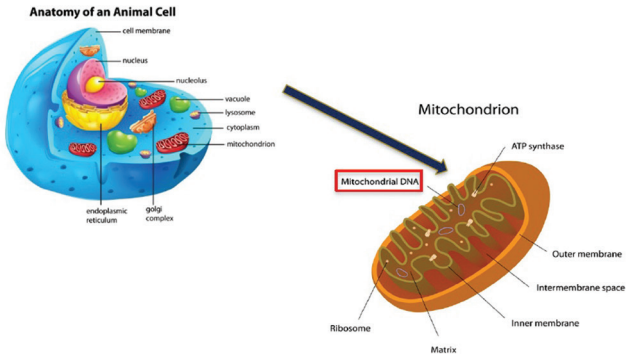

Figure 1. Left: Schematic diagram of multiple mitochondria within a cell. Right: Detailed structure of mitochondrion showing different compartments and location of mtDNA.

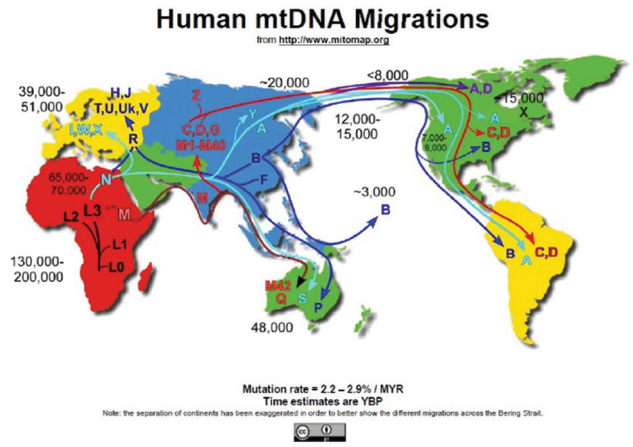

Figure 2. Schematic of map showing origins of different human mtDNA haplogroups, migration patterns and time estimates. From https://www.mitomap.org.

apoptosis (programmed cell death) and retrograde signaling. Retrograde signaling demonstrates that mitochondria transmit signals to the nucleus and thus can regulate nuclear gene expression and cellular behavior. Hence the 'older' idea that the nucleus is the 'big boss' and mitochondria are only involved in the production of ATP has changed significantly. Retrograde signaling (from mitochondria to nucleus) can regulate pathways related to complement, inflammation, angiogenesis, innate immunity, and, which are associated with development and progression of age-related macular degeneration (AMD).[7]

Variations in mitochondrial DNA sequence, called haplogroups, have happened over 150,000 years and are connected to geographic ancestry of distinct populations. It is known that the oldest haplogroups (L haplogroup) originated in Africa and other haplogroups were formed through migration and climate adaptations [Figure 2]. Single nucleotide polymorphism (SNP) variants define the diverse haplogroups (populations). As a result of difference in the mtDNA profiles for different racial/ethnics groups, these SNP changes affect the rates of mtDNA replication and transcription. Moreover, different haplogroup SNP patterns can change the levels of oxidative phosphorylation, which in turn cause variations in ROS production, apoptosis and cell death. Specific haplogroups are related to a wide range of age-related diseases, such as Parkinson's disease, Alzheimer's disease and AMD.[8-13] AMD has been associated with haplogroups that corresponds to Northern European haplogroups, e.g., J, T, and U.[14-17] Those with H haplogroup mtDNA have a protection against AMD.[18] In one study, large soft drusen and pigment abnormalities have been connected to J and U haplogroups.[14] An independent predictor for AMD is related to the SNP defining the haplogroup T, which is in the NADH subunit 2 of complex I.[19] Two SNP variants, associated with the T haplogroup, are located in respiratory complex I and were 2.5 times more likely to be associated with advanced AMD than the age-matched controls.[16]

MITOCHONDRIA AND AGE-RELATED MACULAR DEGENERATION

Human retinal pigment epithelium (RPE) study by transmission electron microscopy has shown that mitochondria are damaged, fragmented and disrupted in AMD.[20] Those findings have been further confirmed by immunohistochemistry in AMD retinas. Karunadharma et al have reported the severity of AMD is linked to a higher number of mtDNA lesions and fragmentations in RPE cells.[21] There was also less nDNA damage with no correlation with AMD severity. Terluk et al study showed that mtDNA damage in AMD presents in RPE cells and not in the neural retina.[22]

TRANSMITOCHONDRIAL CYBRID MODEL TO STUDY AGE-RELATED MACULAR DEGENERATION MITOCHONDRIA

We have created a transmitochondrial cybrid model in our lab to investigate the role of mitochondria in AMD. Cybrids are cell lines that have identical nuclei but mitochondria from different individuals. In our studies, ARPE-19 cells which are an established human RPE cell line were treated to remove their natural mitochondrial DNA, yielding *Rho0 cells*. Then, platelets were isolated from patients with AMD and age-matched control subjects. Platelets are used due to their large numbers of mitochondria without nuclei. Then, the platelets were fused with the *Rho0* ARPE-19 cells devoid of mitochondria and cell lines were established. With this method, different cybrid cell lines were created which all have identical nuclei but mitochondria from patients with wet

AMD, dry AMD or age-matched controls [Figure 3]. In this way, any differences in the molecular or functional behavior of the cybrids can be attributed to mitochondrial influence. In addition, we can correlate the clinical pictures such as type of AMD, response to medication, family history, etc. with the *in vitro* cybrid findings. For example, when cybrids were cultured and stained with the green fluorescent protein that targets mitochondria, we noticed that the mitochondria originating from control subjects were much healthier than those from patients with AMD.[23] The cybrids with J haplogroup mtDNA (high risk for AMD) had significantly lower levels of ATP and reactive oxygen/nitrogen species production, but showed increased lactate levels.[24] Quantitative real-time polymerase chain reaction (qRT-PCR) analyses showed J cybrids had decreased expressions for CFH, C3, and EFEMP1 genes which are the high risk genes for AMD. Alternatively, the growth rates of J cybrids were significantly higher than H cybrids.[24] Another study showed decreased gene and protein expression levels of complement inhibitors along with higher levels of complement activators in AMD cybrids, compared to older-normal cybrids.[23]

Mechanisms by which gene expression and cellular functions are modified without changes in the gene sequence are "epigenetics." Epigenetic factors are reversible and related to the environmental factors, but can be transferred to the next generations. The most common epigenetic changes occur by methylation of the cytosine at the 5 position or modifications of histones through methylation, acetylation, and/or phosphorylation. These epigenetic changes can lead to activation or inhibition of transcription, which regulate the gene expression.[25] DNA methylation levels are modified in cells with depleted mitochondria.[26] Besides, cybrids containing J haplogroups (high risk for AMD) have elevated total methylation levels in comparison with cybrids with H haplogroup mtDNA.[27,28] Further investigations into the role of epigenetics can potentially address new strategies and approaches for the treatment of AMD.

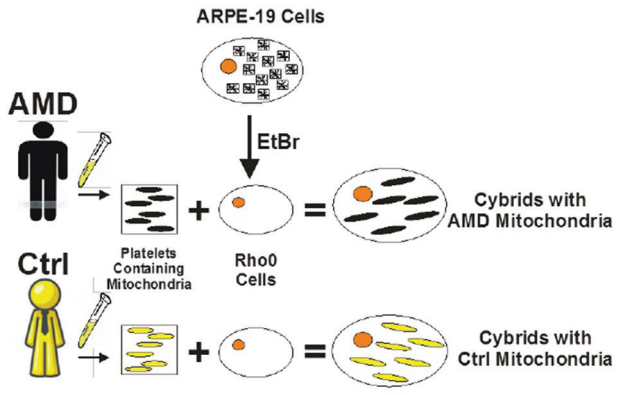

Figure 3. Schematic showing the creation of transmitochondrial cybrids by fusion of platelets. (originating from AMD or control subjects) with *Rho0* RPE cells devoid of mtDNA.

TARGETING MITOCHONDRIA FOR TREATMENTS OF AGING DISEASES

At least two different routes exist to protect the mitochondria. One of them is to act on endogenously produced compounds (such as Humanin) and the other is to target particular pathways engaged in retaining the mitochondrial functions. The Humanin gene (*MT-RNR2*) is located in the 16S rRNA gene of the circular mtDNA. Humanin is a 24 amino acid peptide that has anti-apoptotic and neuroprotective characteristics.[29-33] Aging causes decreased levels of Humanin in mice and human, and led to the assumption that low levels of Humanin may play a crucial role in age-related diseases.[29,34] Also, it has been shown that this gene has been protective in models for Alzheimer's disease, atherosclerosis, heart and brain ischemia and type I diabetes.[34-36] Likewise, Humanin has protective effects against hypoxia-induced toxicity in retinal ganglion cells.[37]

Higher oxidative stress and ROS levels are associated with a decrease in mitochondrial function. Therefore, antioxidant medications such as resveratol and memantine have been used for their protective effects with some promising outcomes.[38,39] Vitamin/mineral supplements that slowed the progression of AMD support the theory that suppressing ROS damage would be an applicable AMD management.[40-43] Other strategies include using substrates or regulators of energy metabolism (e.g., creatine, coenzyme Q10, or quinone analogues), preventing apoptosis by stabilizing mitochondrial permeability using drugs such as cyclosporin A, or inhibiting the mitochondrial fission protein Drp1 with the agents such as MDIV-1.[44-46] The field of mitochondria targeting drugs to treat retinal diseases, such as AMD, is a novel era with exciting capacity to be developed in the future.

In summary, the mitochondria from AMD patients are significantly damaged and may act as biomarkers for this disease. *In vitro* testing of relevant gene expression of AMD cybrids can potentially predict the outcome and response to treatment. Models can potentially be used to find the pharmacotherapeutic agents which may protect against AMD induced mtDNA damage.

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Conflicts of Interest

There are no conflicts of interest.

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