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Increased expression of ApoE and protection from amyloid-beta toxicity in transmitochondrial cybrids with haplogroup K mtDNA

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ABSTRACT

Mitochondrial (mt) DNA haplogroups, defined by specific single nucleotide polymorphism (SNP) patterns, represent populations of diverse geographic origins and have been associated with increased risk or protection of many diseases. The H haplogroup is the most common European haplogroup while the K haplogroup is highly associated with the Ashkenazi Jewish population. Transmitochondrial cybrids (cell lines with identical nuclei, but mtDNA from either H ($n = 8$) or K ($n = 8$) subjects) were analyzed by the Seahorse flux analyzer, quantitative polymerase chain reaction (Q-PCR) and immunohistochemistry (IHC). Cybrids were treated with amyloid-β peptides and cell viabilities were measured. Other cybrids were demethylated with 5-aza-2′-deoxycytidine (5-aza-dC) and expression levels for *APOE* and *NFkB2* were measured. Results show K cybrids have (a) significantly lower mtDNA copy numbers, (b) higher expression levels for MT-DNA encoded genes critical for oxidative phosphorylation, (c) lower Spare Respiratory Capacity, (d) increased expression of inhibitors of the complement pathway and important inflammasome-related genes; and (e) significantly higher levels of *APOE* transcription that were independent of methylation status. After exposure to amyloid-β_{1-42} peptides (active form), H haplogroup cybrids demonstrated decreased cell viability compared to those treated with amyloid-β_{42-1} (inactive form) ($p < 0.0001$), while this was not observed in the K cybrids ($p = 0.2$). K cybrids had significantly higher total global methylation levels and differences in expression levels for two acetylation genes and four methylation genes. Demethylation with 5-aza-dC altered expression levels for *NFkB2*, while *APOE* transcription patterns were unchanged. Our findings support the hypothesis that mtDNA-nuclear retrograde signaling may mediate expression levels of APOE, a key factor in many age-related diseases. Future studies will focus on identification of the mitochondrial-nuclear retrograde signaling mechanism(s) contributing to these mtDNA-mediated differences.

Abbreviations: 5-aza-dC, 5-aza-2′-deoxycytidine; AD, Alzheimer's disease; AMD, Age-related Macular Degeneration; APOE, apolipoprotein E; ARPE-19, retinal pigmented epithelium cell line; ATP, adenosine triphosphate; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; DNA, deoxyribonucleic acid; ECAR, extracellular acidification rate; EDTA, ethylenediaminetetracetic acid; ETC, electron transport chain; FCCP, carbonyl cyanide 4-trifluoromethoxy-phenylhydrazone; IHC, immunohistochemistry; LDL, low density lipid; LHON, Leber hereditary optic neuropathy; μM, micromolar; MDP, mitochondrial derived peptide; Mt, mitochondrial; MT-CYB, mitochondria encoded cytochrome B; MT-ND1, mitochondria encoded NADH dehydrogenase 1; MT-ND4, mitochondria encoded NADH dehydrogenase 4; MT-ND5, mitochondria encoded NADH dehydrogenase 5; MT-ND6, mitochondria encoded NADH dehydrogenase 6; MT-CO1, mitochondria encoded cytochrome oxidase 1; MT-CO2, mitochondria encoded cytochrome oxidase 2; MT-CO3, mitochondria encoded cytochrome oxidase 3; MT-ATP6, mitochondria Encoded ATP synthase 6; MT-ATP8, mitochondria Encoded ATP synthase 8; *NFkB2*, nuclear factor of kappa light polypeptide gene enhancer in B-cells 2; OCR, oxygen consumption rate; OXPHOS, oxidative phosphorylation; Q-PCR, quantitative polymerase chain reaction; PCR, polymerase chain reaction; Rho*0*, lacking mtDNA; ROS, reactive oxygen species; RPE, retinal pigment epithelial; SEM, standard error mean; SNPs, single nucleotide polymorphisms; $VO2_{max}$, maximal oxygen uptake.

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1. Introduction

Mitochondria (mt) are unique organelles with circular, double-stranded DNA containing 16,569 nucleotide pairs. The coding region of mtDNA encodes for 37 genes, including 13 protein subunits essential for oxidative phosphorylation (OXPHOS), 2 ribosomal RNAs and 22 transfer RNAs. (Wallace, 1992, 1994; McFarland and Turnbull, 2009) The non-coding region of 1121 nucleotides, known as the MT-Dloop, is critical for mtDNA replication and transcription. Most recently, it has been reported that biologically active mitochondrial-derived peptides (MDPs) are encoded from the *16s* and *12s* rRNA of the mtDNA. (Lee et al., 2015; Yen et al., 2013) Geographic origins of populations can be classified into haplogroups based upon the patterns of accumulated single nucleotide polymorphisms (SNPs) with the mtDNA.

Studies show that mtDNA haplogroups can confer either increased risk or protection for many human diseases, including Alzheimer's disease (AD) and age-related macular degeneration (AMD). (Wallace et al., 2007; Czarnecka and Bartnik, 2011; Ridge et al., 2012; Strauss et al., 2013; De Luca et al., 2012; Fernandez-Caggiano et al., 2013; Canter et al., 2006; Fernandez-Caggiano et al., 2012; Fesahat et al., 2007; Bi et al., 2015; Jones et al., 2007; Canter et al., 2008; Udar et al., 2009; SanGiovanni et al., 2009; Mueller et al., 2012a; Kenney et al., 2013a) Both Alzheimer's disease and AMD are associated with inflammation, oxidative stress, specific ApoE allele profiles and amyloid-β deposits, along with risk factors including smoking, obesity, elevated cholesterol, hypertension and aging. (Kaarniranta et al., 2011; Zhao et al., 2015; Hyttinen et al., 2014) In these diseases, mitochondrial dysfunction, retrograde signaling, and epigenetic abnormalities altering nuclear gene expression also contribute to their pathogenesis. (Salminen et al., 2015; Hjelmeland, 2011; Guha and Avadhani, 2013; Whelan and Zuckerbraun, 2013) Therefore, intense interest has developed to understand the underlying mechanisms of these intricate mitochondrial-nuclear interactions.

A major hurdle in identifying the effects of mtDNA upon cellular homeostasis is the variability of nuclear genes from one individual to another. This problem can be addressed by using the transmitochondrial cybrid models, which are cell lines with identical nuclei, but the mtDNA from different subjects. Using the cybrid model, it has been demonstrated that different mtDNA haplogroups mediate cellular bioenergetics, the levels of methylation, rates of growth, and transcription of inflammatory, complement and signaling pathway genes. (Bellizzi et al., 2009; Gomez-Duran et al., 2010; Chen et al., 2012; Pacheu-Grau et al., 2013; Kenney et al., 2013b, 2014a, 2014b; Malik et al., 2014) In addition, cybrids with different mtDNA haplogroups have different responses to hydrogen peroxide or ultraviolet radiation. (Malik et al., 2014; Mueller et al., 2012b; Lin et al., 2012) The conplastic mouse model, which crosses the mtDNA from one strain of mouse into a different background, has illustrated altered mitochondrial-nuclear interactions and increased susceptibility to cardiovascular disease. (Fetterman et al., 2013) These studies support the hypothesis that an individual's mtDNA background contributes to baseline cellular homeostasis, making the cells differentially susceptible to identical stressors and contributing to differential disease susceptibility.

The UK cluster, comprised of both the U and K haplogroups, is defined by the A12308G SNP. The group diverges with the G9055A that defines the K haplogroup. The K mtDNA haplogroup (also known as Uk) has a 1–6% worldwide distribution (www.MitoMap.org). Approximately 10% of the ancestral Europeans fall within the K haplogroups. One group highly associated with the K haplogroup is the Ashkenazi Jewish population, which is defined as K1a1b1a, K2a2a and K1a9 subsets. (Behar et al., 2004) Within the Ashkenazi Jewish population, approximately 32% can be classified in the K haplogroup, a high percentage that has occurred due to a genetic bottleneck. (Behar et al., 2004) The H haplogroups are the most common European mtDNA haplogroup (www.mitomap.com). Those individuals of maternal African-origin possess the L haplogroup, which is the oldest and most diversified haplogroup. The diverse racial/ethnic populations have different risks for specific diseases. For example, African-Americans are susceptible to developing type 2 diabetes, obesity and prostate cancer. (Hatzfeld et al., 2012; Mensah et al., 2005; Kurian and Cardarelli, 2007) The Ashkenazi Jews are prone to having high levels of cholesterol and lipid along with other cardiovascular diseases at young ages. (Seftel et al., 1989; Jenkins et al., 1980) Scientifically, when studying the relationship between genetics and specific diseases, it is helpful to study well-defined, homogeneous populations so genetic changes can more easily be identified. (Guha et al., 2012) As a successful example of this approach, it was the examination of Ashkenazi Jews females that led to the identity of *BRCA1* and *BRCA2* genes being associated with breast and ovarian cancers. (Lancaster et al., 1997; Berchuck et al., 1998).

The Ashkenazi Jewish population is an excellent model to study age-related diseases because the number of founders is limited, there have been population bottlenecks (sharp reduction in population size due to environmental or sociological events) and the population tends to marry within their communities. (Atzmon et al., 2005) As populations age, then the likelihood for individuals to develop age-related diseases is increased. In the present study, we illustrate that although the human retinal pigment epithelial (RPE) cybrids all have identical nuclei, the cybrids with K haplogroup mtDNA have: (1) significantly increased expression of ApoE, a critical lipid transporter molecule associated with human diseases; (2) higher degree of protection from cytotoxic effects of amyloid-β_{1-42} (active form); (3) increased expression of inhibitors of the alternative complement pathways and important inflammasome-related genes; and (4) elevated bioenergetic respiratory profiles compared to the H cybrids. These findings suggest that an individual's mtDNA may, by as of yet unknown mechanism(s), contribute to lipid transport, cholesterol metabolism, complement activation and inflammation, factors critical for AMD, Alzheimer's disease and other age-related diseases.

2. Materials and methods

2.1. Cybrid cultures and culture conditions

Institutional review board approval was obtained from the University of California, Irvine (*#2003-3131*).Peripheral blood was collected in tubes containing sodium citrate and DNA was isolated with a DNA extraction kit (PUREGENE, Qiagen, Valencia, CA) and quantified using Nanodrop 1000 (Thermo Scientific, Wilmington, DE). Platelets were isolated by a series of centrifugation steps and final pellets were suspended in Tris buffer saline (TBS). ARPE-19 cells, are a human diploid cell line showing structural and functional properties similar to RPE cells in vivo, derived from human retinal epithelia (ATCC, Manassa, VA). (Dunn et al., 1998) The ARPE-19 cells were made Rho*0* (deficient in mtDNA) by serial passage in low dose ethidium bromide. (Miceli and Jazwinski, 2005) Cybrids were produced by polyethylene glycol fusion of platelets with Rho*0* ARPE-19 cells as described previously.(Kenney et al., 2013b) Cybrids were cultured until confluent in DMEM-F12 containing 10% dialyzed fetal bovine serum, 100 unit/mL penicillin and 100 μg/mL streptomycin, 2.5 μg/mL fungizone, 50 μg/mL gentamycin and 17.5 mM glucose. A total of 11 haplogroup H cybrids were produced and 10 haplogroup K cybrids were cultured to passage 5 for the experiments. The age, gender, and sub-haplogroup data are described in Table 1.

2.2. Inhibition of methylation in cybrid cultures

Experiments were performed to determine if inhibition of methylation sites in the K and H cybrids might affect the RNA expression for nuclear genes associated with age-related diseases. The H cybrids ($n = 3$) and K cybrids ($n = 3$), each from different individuals, were plated for 24 h, media were removed and replaced with the same media containing