Mitochondrial Dysfunction in FECD

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Fuchs endothelial corneal dystrophy (FECD) is a genetically complex, heterogenous, age-related degenerative disease of corneal endothelial cells (CEnCs), occurring in the fifth decade of life with a higher incidence in females. It is characterized by extracellular matrix (ECM) protein deposition called corneal guttae, causing light glare and visual complaints in patients. In FECD, CEnCs exhibit stress-induced senescence, oxidative stress, DNA damage, heightened reactive oxygen species (ROS) production, mitochondrial damage, and dysfunction as well as sustained endoplasmic reticulum (ER) stress. Among all of these, mitochondrial dysfunction involving altered mitochondrial bioenergetics and dynamics plays a critical role in FECD pathogenesis.

Keywords: CEnCs ; mitochondrial dysfunction ; DNA damage ; mitophagy ; FECD

1. Introduction

The corneal endothelial is the innermost layer of the cornea and plays an important role in maintaining water balance and clarity of the cornea. Fuchs endothelial corneal dystrophy (FECD) is the most common corneal endothelial dystrophy. It is a bilateral, genetically heterogeneous degenerative disease of CEnCs occurring in 4% of the U.S. population over 40 years of age, with a higher incidence in women [1][2][3][4]. It is characterized by the progressive decline of the CEnCs and the formation of extracellular matrix excrescences [5][6][7] in Descemet's membrane (DM), called guttae, leading to corneal edema and loss of vision. Currently, the only treatment for FECD is corneal transplantation, which accounts for approximately 32,000 of the endothelial keratoplasties performed in the U.S. annually. It carries substantial economic and social burdens. Understanding the disease pathogenesis is essential for developing pharmacotherapeutic interventions to halt the disease. In FECD, CEnCs exhibits stress-induced senescence [8], oxidant-antioxidant imbalance [9], mitochondrial DNA damage [10] and dysfunction [11], sustained unfolded protein response (UPR) [12][13], and endoplasmic reticulum (ER) stress [14]. Among these factors, mitochondrial stress [15][16][17][18] plays an important role in FECD pathogenesis. Maintaining functional mitochondria is the key to the ion pump function in CEnCs. Excessive damage to the mitochondria leads to its selective degradation called auto(mito)phagy [15][18].

2. Mitochondria in CEnCs

Due to many ion pumps and very active endothelial cell metabolism, mitochondrial density is very high in CEnCs, second only to retinal photoreceptors [19]. Mitochondria, the powerhouse of the cells, regulate many physiological processes in CEnCs and play a pivotal role in their survival [20][21][22].

3. Mitochondrial DNA Damage in FECD

Mitochondrial DNA (mtDNA) damage occurs in many neurodegenerative disorders [23] such as Alzheimer's, Parkinson's, and Huntington's diseases. It also occurs in retinal diseases [24] such as age-related macular degeneration, diabetic retinopathy, glaucoma, and in corneal diseases [25] such as keratoconus, Kearns Sayre Syndrome, and FECD [26][27]. In human FECD tissues, we found that 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidized DNA lesions, accumulated in mtDNA of CEnCs, suggesting increased oxidative mtDNA damage [9]. Further studies using long-amplicon-quantitative polymerase chain reaction (LA-qPCR) demonstrated that human FECD specimens had significantly decreased small mitochondrial copy number and increased DNA lesion frequency (indicative of damage) than the normal specimens [11].

As Fuchs is prevalent in females [2][3], Liu et al. analyzed mtDNA damage in the mouse model of ultraviolet A (UVA)-induced FECD for both sexes and found that mtDNA damage was significantly more in the mouse corneal endothelial cells (MCEnCs) at week 4 and 8 post-UVA in females compared to males, suggesting the female susceptibility to FECD [10]. However, mtDNA exhibited a similar extent of damage in both male and female mice at day 1 post-UVA, and it recovered at week 2 post-UVA [10].

For the in vitro studies, menadione (MN) induced oxidative stress to study mtDNA damage in normal (HCEnC-21T or HCECi: normal telomerase immortalized human corneal endothelial) and Fuchs (FECDi: immortalized FECD cell lines derived from FECD specimens) cell lines. Halilovic et al. demonstrated that the FECD cell line had significantly reduced the small mtDNA copy number compared to the normal control cell line and remained low in quantity after MN exposure [11]. MtDNA damage was significantly greater in the control cell lines than the untreated control and remained significantly high in the FECD cell line after MN exposure [11]. Miyajima et al. demonstrated a dysfunctional Nrf2-NQO1 axis, and specifically loss of NQO1 (NAD(P)H:quinone oxidoreductase 1) protein in FECD contributes to mitochondrial DNA damage and estrogen genotoxicity, explaining the higher incidence of FECD in females [28].

4. Mitochondrial Dysfunction in FECD

Mitochondrial stress and dysfunction [29][30][31] have been involved in many diseases such as neurodegenerative diseases [32], cancer [33], and diabetes [34]. It is central to aging processes [35] and critical for the degeneration of post-mitotic cells in other organs [36], similar to non-replicative CEnCs in FECD [37]. In general, mitochondrial dysfunction in FECD comprises aberrant mitochondrial bioenergetics and dynamics. Abnormal mitochondrial bioenergetics in FECD involves loss of mitochondrial membrane potential (MMP) [15], abnormal ATP production [11], and increased production of mitochondrial ROS [11]. Mitochondrial dynamics consists of a coordinated cycle of mitochondrial fusion and fission to maintain its shape and size. Abnormal mitochondrial dynamics in FECD comprises aberrant mitochondrial fusion including decline in mitofusin 2, loss of mitochondrial mass [15][16], and fission including increased mitochondrial fission proteins indicative of fragmentation [18], and activation of mitochondrial-mediated intrinsic apoptosis pathway [11].

Specifically, with regard to mitochondria bioenergetics, the central FECD human corneal tissue samples showed reduced cytochrome oxidase activity (a complex IV enzyme of the mitochondria electron transport chain), clinically associating with central corneal edema [38]. Similarly, FECD cell lines demonstrated the absence of electron transport subunits, complex I and V involved in mitochondrial bioenergetics [15]. Decreased/absent mitochondrial electron transport chain proteins in FECD may initiate the alteration in MMP. FECD cell lines showed increased susceptibility to MMP loss in response to the mitochondrial depolarization agent, m-chlorophenyl hydrazone (CCCP) [15]. Loss of MMP could lead to abnormal mitochondria bioenergetics, triggering abnormal ATP production, oxygen consumption, and increased mitochondria ROS in FECD. Specifically, Halilovic et al. demonstrated a significant decrease in MMP for the Fuchs cell line compared to normal control cell lines at the baseline. The Fuchs cell line demonstrated a further decrease in MMP after treatment with MN compared to normal control cell lines [11].

Concerning aberrant mitochondria dynamics in FECD, Halilovic et al. also demonstrated mitochondrial fragmentation demonstrated by cytochrome c release in human Fuchs specimen compared to the normal control [11]. MN significantly increased cytochrome c release with time in the normal control cell line compared to untreated cells [11]. Mitochondrial fragmentation might lead to loss of mitochondrial mass in FECD.

Despite several studies on mitochondrial DNA damage and dysfunctional mitochondria in FECD, there has not been any study directly associating the two phenomena in FECD. However, in other ocular [24] and neurological diseases [23], mitochondrial DNA damage is associated with mitochondrial dysfunction and disease pathogenesis. Based on mitochondrial dysfunction in many ocular and neurological diseases, mtDNA damage can be one of the major contributing factors for mitochondrial dysfunction in FECD.

5. Autophagy and Mitophagy

Mitophagy is a specialized and evolutionarily conserved autophagy involving degradation of damaged mitochondria, which accumulates following stress, thus regulating mitochondrial turnover and quality control and health. Mitochondria quality control is a complex cellular protective mechanism and occurs through the coordination of many cellular processes such as proteostasis, biogenesis, and dynamics including mitophagy to maintain cell homeostasis. Imbalance of mitochondrial quality control occurs in many diseases in response to mtDNA damage upon various intracellular or extracellular stress. Under extreme stress, when there is minimum possibility of maintaining cell homeostasis through mitochondria quality control, cells activate their apoptotic pathways to control cell fate.

6. Mechanisms of Mitophagy in FECD

Few studies have demonstrated the role of mitophagy in the pathogenesis of FECD [15][39] (**Figure 1**). Specifically, Benischke et al. demonstrated abnormal, swollen mitochondria in vacuoles called autophagosomes, suggesting initial signs of mitophagy in FECD human tissues using transmission electron microscopy (TEM) [15].

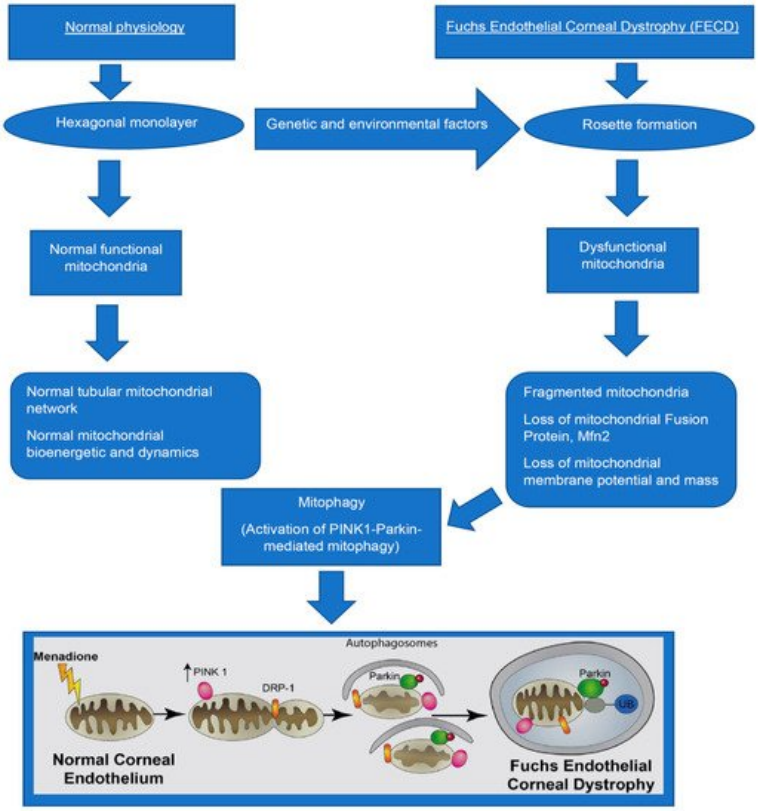

Figure 1. Mitophagy in Fuchs endothelial corneal dystrophy. Schematic diagram demonstrating the formation of dysfunctional mitochondria that leads to mitophagy in Fuchs. In FECD, various genetic and environmental factors lead to the conversion of hexagonal CEnCs into an irregular shape, thereby resulting in rosette formation. Subsequently, there is mitochondria DNA damage, fragmentation, loss of mitochondria fusion protein (Mfn2), mass and membrane potential, which lead to activation of PINK1-Parkin mediated mitophagy, thereby resulting in clearance of abnormal mitochondria.

In general, mitophagy could be driven by the loss of MMP, electron transport chain proteins, and mitochondrial dysfunction, as seen in FECD as described in the previous section. This could lead to a significant decline in mitochondrial mass as seen in the FECD cell line compared with the control cell line (HCECi) [15] at the baseline. Moreover, CCCP could lead to a significant decline in mitochondrial mass in the control cell line (HCECi) [15]. Loss of mitochondria mass could directly contribute to mitophagy in FECD. In conclusion, autophagy in mitochondria or mitophagy drives the loss of Mfn2 in FECD [15].

Another molecular mechanism for mitophagy in FECD is PINK1(PTEN-induced putative kinase 1)-Parkin pathway activation, as described by our group [18]. In the PINK1-Parkin mediated mitophagy, there was upregulation of PINK1 in the outer mitochondrial membrane, which recruits the cytosolic E3 ubiquitin ligase Parkin and ubiquitin by phosphorylating Serine65 in both proteins. Parkin translocate to the mitochondria, and further ubiquitinates its substrates to proteasomal degradation. The ubiquitination also initiates the recruitment of phagosomes to mitochondria, removing them through the autophagosomal pathway. Miyai et al. showed that human FECD samples demonstrated upregulation of PINK1, phosphor-Parkin with a decrease in total Parkin [18]. A similar observation was seen at least for phosphor-Parkin and Parkin in normal cell lines after the MN induced mitochondrial fragmentation in vitro [18]. FECD cell lines also demonstrated a significant decrease in the total Parkin with significant upregulation of the phosphor-Parkin (Ser65) compared to the control cell line when treated with CCCP. To investigate the ubiquitination and proteasomal degradation of mitochondrial following Parkin recruitment, a proteasomal inhibitor (Epoxomicin) was used and demonstrated increased fold in the rescue of total Parkin after treatment of CCCP in the Fuchs cell line compared to the untreated control cell line. This suggests a possible abnormal stimulation of proteasome-mediated degradation in FECD.

7. Role of Mitophagy in FECD

The activation of mitophagy being detrimental or useful remains unclear in FECD. PINK1-Parkin mediated mitophagy, as described by our group, suggests that excessive mitophagy might destroy many normal mitochondrial, disturbing mitochondrial bioenergetics, and contributing to the cascade of destructive events in the pathogenesis of FECD. Therefore, therapeutics targeting mitophagy might not be beneficial in the late stage of FECD. However, it might be advantageous in the earlier stages of FECD as CEnCs might alleviate some intracellular mitochondrial stress by removing damaged mitochondria via mitophagy.

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