The Importance of Mitochondria in Age-Related and Inherited Eye Disorders

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Key Words

Mitochondria • Ocular function • Reactive oxygen species

Abstract

Mitochondria are critical for ocular function as they represent the major source of a cell's supply of energy and play an important role in cell differentiation and survival. Mitochondrial dysfunction can occur as a result of inherited mitochondrial mutations (e.g. Leber's hereditary optic neuropathy and chronic progressive external ophthalmoplegia) or stochastic oxidative damage which leads to cumulative mitochondrial damage and is an important factor in age-related disorders (e.g. age-related macular degeneration, cataract and diabetic retinopathy). Mitochondrial DNA (mtDNA) instability is an important factor in mitochondrial impairment culminating in age-related changes and pathology, and in all regions of the eye mtDNA damage is increased as a consequence of aging and age-related disease. It is now apparent that the mitochondrial genome is a weak link in the defenses of ocular cells since it is susceptible to oxidative damage and it lacks some of the systems that protect the nuclear genome, such as nucleotide excision repair. Accumulation of mitochondrial mutations leads to cellular dysfunction and increased susceptibility to adverse events which contribute to the pathogenesis of numerous sporadic and chronic disorders in the eye.

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Introduction

Accumulating evidence supports a role for mitochondrial dysfunction in aging and disease in a wide range of tissues resulting in sporadic and chronic disorders, including neurodegeneration and cardiomyopathy [1, 2]. It is now apparent that the ocular system is no exception, since numerous eye pathologies are associated with mitochondrial defects as a result of inherited mitochondrial mutations (e.g. Leber's hereditary optic neuropathy, LHON) or cumulative stochastic mitochondrial damage (e.g. age-related macular degeneration, AMD; diabetic retinopathy). Cumulative stochastic injury over a lifetime is not surprising, since the eye is constantly exposed to numerous damaging agents including visible light (in particular the blue region, 475–510 nm), UV (UVA, 320–400 nm) and UVB (280–320 nm) radiation, high concentrations of oxygen, and environmental chemicals (e.g. acrolein from tobacco smoke). This potentially damaging microenvironment strongly favors the generation of reactive oxygen species (ROS), which have the potential to exacerbate mitochondrial mutagenesis and ocular dysfunction [3, 4]. As will be discussed below, cellular ROS can be derived from three main sources: mitochondria, photosensitizers and NADPH oxidase. Elevated mitochondrion-derived ROS have been strongly associated with ocular diseases in both the anterior and posterior

segments of the eye [3, 5–7]. In addition, inherited mutations in either mitochondrial genes or nuclear genes encoding mitochondrial proteins result in severe mitochondrial disease causing respiratory chain dysfunction, impairment of replication of mitochondrial DNA (mtDNA) and depletion of mtDNA, which often manifest themselves as disorders of the optic nerve [8–10].

The mitochondrion represents a critical organelle for cellular function and survival. Its principal roles include generation of chemical energy, compartmentalization of cellular metabolism and regulation of programmed cell death. The mitochondria consist of inner and outer membranes composed of phospholipid bilayers containing many integral proteins. The inner membrane is particularly protein rich and is compartmentalized into an inner limiting membrane and complex invaginations designated ‘cristae’ that expand the surface area for ATP production [11]. Encompassed by the inner membrane is the matrix that contains enzymes catalyzing the tricarboxylic acid and urea cycles, fatty acid oxidation, gluconeogenesis as well as committed steps in heme and amino acid synthesis. In addition, the mitochondrial matrix houses the genetic system of the organelle: ribosomes, transfer RNAs (tRNAs) and tRNA synthetases, multiple copies of the mtDNA and the enzymes required for its replication. Given the fact that mitochondria play such an integral role in cellular activity, it is perhaps not surprising that dysfunction can result in a myriad of clinical disorders arising from inherited mutations and/or stochastic ROS-induced genomic injury (fig. 1).

In this review, we outline the mechanistic basis of ROS-induced mtDNA dysfunction and the mitochondrial/oxidative stress theory of aging in relation to the ocular system. In addition, we will discuss the common pathologies associated with mitochondrial dysfunction, with particular emphasis on the role of oxidatively induced and inherited mutations within mtDNA, as causative factors in aging and tissue dysfunctions of the eye.

Mitochondrial and Free Radical Theories of Aging and Disease

The free radical theory of aging was first proposed over 60 years ago, when it was discovered that oxygen radicals were generated in biological systems following exposure to radiation [12, 13]. Harman [14] put forward that oxygen free radicals are formed endogenously during routine metabolism and that they play a pivotal role in the aging process. The discovery of oxidants in vivo and the identifica-

tion of superoxide dismutase (SOD), an antioxidant whose primary function is to prevent oxidative damage in cells, propelled this principle to the forefront of gerontology theories [15, 16]. In 1972, Harman [17] additionally proposed that mitochondria have a fundamental role in the process of aging. The overall premise of this theory was that ROS generated by the mitochondrial metabolism increase in an age-dependent manner and that ROS-mediated damage to proteins, lipids and mtDNA has a causative role in the morbidity of aging and age-related disease. There are other plausible theories of senescence involving, for example, telomeres and genome stability, but the discovery of the role of sirtuin proteins (a family of proteins that regulate gene silencing and suppress recombination of ribosomal DNA) on mitochondrial function and on extension of life span has refocused attention on the importance of mitochondria in aging [18]. Recent research has demonstrated that mitochondrial deficiencies (either generated by stochastic damage or inherited mutations) are an important cause of cellular degeneration that is associated with ocular dysfunction and aging and have led to the ‘mitochondrial-mutation-based’ model of ocular degeneration (fig. 2) [19–22]. The cumulative effects of these oxidatively modified mitochondrial components reduce the bioenergetic tone, impair DNA repair, promote ROS production and increase the mtDNA mutation rate.

Inherited Mitochondrial Diseases

The best understood mitochondrial diseases are caused by maternally inherited point mutations or deletions in mtDNA. In addition, mutation of nuclear encoded genes can result in loss of mtDNA stability [23]. Mitochondrial diseases manifest themselves with a broad spectrum of symptoms but frequently affect ocular tissues and often involve heteroplasmy (the coexistence of both mutant and normal mtDNA; fig. 1). Examples include cases of LHON, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), chronic progressive external ophthalmoplegia (PEO), and neuropathy, ataxia and retinitis pigmentosa. The symptoms of these disorders range from ophthalmoplegia, optic atrophy, pigmentary retinopathy to macular dystrophy [24].

mtDNA Heteroplasmy

A single mitochondrion may contain 0–21 mtDNA molecules [25, 26]. In healthy cells, the mtDNA molecules are identical, which is termed homoplasmy. Most homo-

Fig. 1. The role of mtDNA (a), nuclear DNA (nDNA) mutations (b) and ROS (c) in mitochondrial disease. Multiple factors diminish the integrity of mitochondria that lead to loss of cell function, apoptosis and ocular degeneration. **a** The most common mitochondrial diseases, e.g. LHON, result from primary mtDNA mutations that prevent successful completion of respiratory complexes, e.g. complex I, thus reducing mitochondrial oxidative phosphorylation (OXPHOS). **b** Mutations in nDNA-encoded mitochondrial proteins result in an impaired ability to undergo mtDNA replication, maintenance and mtDNA repair. **c** ROS, in particular superoxide ($O_2^{\cdot-}$), are generated from exposure to exogenous

oxidative agents as well as being byproducts of OXPHOS (primarily from complexes I and III of the electron transport chain). ROS damage all mitochondrial macromolecules and include labile Fe-S enzymes such as aconitase which release Fe^{2+} and H_2O_2 , promoting Fenton chemistry. The mtDNA is a major target for the hydroxyl radical ($OH^{\cdot}$) which can lead to increased mutation rates. It is important to note that increased ROS generation is associated with mitochondrial disease irrespective of the causative factor, i.e. mtDNA, nDNA mutation or exogenous oxidative exposure.

plasmic base substitutions are usually found to be neutral polymorphisms, but some homoplasmic mitochondrial DNA mutations such as G11778A in the ND4 gene are an important cause of ocular disease [27, 28]. However, due to the polyploid nature of the mitochondrial genome, wild-type and mutated mtDNA may coexist in an individual mitochondrion, which is referred to as heteroplasmy. Most mtDNA mutations related to diseases are asso-

ciated with heteroplasmy and can, if within a transcribed mitochondrial gene, affect expression and/or activity of the gene product. In heteroplasmic pedigrees, individuals with a greater amount of mutant mtDNA are at higher risk for visual loss. Typically 80–90% mutant mtDNA is required to produce a clinically observable phenotype. This phenomenon is known as the threshold effect. However, the additional interaction with nuclear-encoded mi-

Fig. 2. Mitochondrial-mutation-based model of ocular degeneration. Both mtDNA mutations and nuclear-DNA (nDNA)-encoded mutations in mitochondrial proteins are a primary cause of mitochondrial dysfunction which may in turn be an initiating factor in ocular disease. Intriguingly, mitochondrial oxidative stress (which can be generated from both endogenous and exogenous sources, as well as being a byproduct from mtDNA and nDNA mutations) has a pivotal role in disease progression. After

a certain threshold of mitochondrial mutations has been reached, the mitochondria undergo a bioenergetic crisis, resulting in increased ROS and concomitant generation of mtDNA mutations. This causes the level of energy production to drop below that required for cellular functioning, and apoptosis is initiated by the mitochondria, leading to loss of tissue function and contributing to the onset/progression of ocular degeneration.

tochondrial proteins and environmental factors may complicate the issue of assigning a pathogenic role specifically to mtDNA sequence variants. These mutations often undergo mitotic segregation and the levels of normal and mutated mtDNA can vary considerably between cells of the same tissue, a phenomenon that may be influenced by the organization of mtDNA in nucleoids. Mitochondrial heteroplasmy is thought to result in altered proteins that may accumulate over time, thus promoting ocular disease states [6, 29].

mtDNA Mutations

The most common mitochondrial disease with bilateral optic neuropathies is LHON, which results from pri-

mary mtDNA mutations affecting the respiratory chain complexes [30, 31]. Over 90% of LHON pedigrees harbor 1 of 3 mtDNA point mutations (m.3460G:A, m.11778G:A, m.14484T:C), all of which affect genes encoding complex I of the respiratory system [32]. Several other mtDNA mutations have been identified with LHON, with most of them also occurring in the respiratory chain [33, 34]. Another mtDNA mutation disorder, MELAS, usually occurs from maternal inheritance, with almost 90% of patients suffering from a mutation at position 3243, encoding the tRNA leucine in mtDNA [35]. Patients with MELAS often have mtDNA deletions and present with retinal pigmentary abnormalities and retinal pigment epithelium (RPE) atrophy similar to those present in the early AMD pheno-

type [36]. Interestingly, similar symptoms have been associated with some patients with another mitochondrial disorder, maternally inherited diabetes and deafness, which highlights the clinical overlap which often exists with mitochondrial diseases [37, 38]. Neuropathy, ataxia and retinitis pigmentosa syndrome is usually due to the ATP6 T8993G mutation. Children in whom the burden of the mutation exceeds 95% instead succumb to a brainstem disorder known as infantile Leigh's syndrome. Mutations in several of the mitochondrial tRNA genes result in PEO, which is characterized by weakness of the external eye muscles and ptosis. The most common mitochondrial deletion, which causes a multisystem disorder known as Kearns-Sayres syndrome, is associated with the development of retinitis pigmentosa and PEO before the age of 20. Patients with Kearns-Sayres syndrome typically suffer from cardiac conduction defects and cardiomyopathy.

Nuclear DNA Mutations That Affect Mitochondria

Mutations in mitochondrial proteins that are encoded by nuclear DNA (nDNA) also present with ophthalmic manifestations that affect POLG encoding DNA polymerase γ , PEO1 or Twinkle encoding the mtDNA helicase and ANT1 encoding the adenine nucleotide translocator [39–42]. These proteins are required for mtDNA replication, maintenance and repair. Over 80 pathogenic mutations have been documented in DNA polymerase γ (a heterotrimer consisting of a catalytic α - and 2 accessory β -subunits) [43]. It is required for DNA repair as a component in the base excision repair pathway and replication [44]. Mutations in its α -subunit can result in disorders that present as ocular symptoms including sensory-atactic neuropathy, dysarthria and ophthalmoplegia (SANDO) and recessive and sporadic forms of PEO [45]. Twinkle, a hexameric DNA helicase, is generally assumed to be the primary replicative helicase of mtDNA with a 5' to 3' polarity and nucleotide triphosphate hydrolase activity. Twinkle interacts with approximately 20 proteins including DNA polymerase γ , to form a component of a replication unit of mtDNA, which helps to explain why mutations in these 2 genes often present in similar manifestations. Over 20 mutations in the Twinkle gene have been associated with PEO, but it has also been described as a recessive mutation in SANDO [46–48]. Mutations in the *ANT1* gene that encodes an adenine nucleotide translocator protein, whose main function is to transport ATP out of the mitochondrial matrix, are also associated with PEO. *ANT1* mutations alter the mitochondrial nucleotide pool which adversely affects mtDNA replication and availability of ADP, thus negatively impacting on the rate

of oxidative phosphorylation. Both familial and sporadic missense mutations of *ANT1* are thought to occur within the transmembrane domains of the protein [49].

Nucleotide Imbalance and mtDNA Depletion

mtDNA depletion syndrome is a recently recognized disorder involving a quantitative defect of mtDNA. It presents in infancy and is associated with mutations in proteins that are predominantly involved in dNTP synthesis (mitochondrial and cytosolic) including TK2 (encoding mitochondrial thymidine kinase), DGUOK (deoxyguanosine kinase) and SUCLA1 and 2 (α - and β -subunit of the mitochondrial matrix enzyme succinyl-CoA synthetase) among others [50–52]. Constant supplies of dNTPs are crucial for the maintenance of mitochondrial genomic integrity, which are either supplied by import of cytosolic dNTPs or by salvaging deoxynucleosides within mitochondria. Alterations in the cellular nucleotide pool generate a promutagenic state and inefficient DNA repair and synthesis propagating mitochondrial mutagenesis [53]. The first identified mitochondrial disease associated with the dNTP pool is mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), which is characterized by ocular defects such as ophthalmoplegia and pigmentary retinopathy [54]. It is of further interest to note that nucleotide imbalance, such as that observed in MNGIE, is highly mutagenic and has the potential to impair the DNA repair processes [55].

Mitochondrially Generated ROS

Mitochondria account for the bulk of endogenously formed ROS in most cells [17, 56, 57] with the mitochondrial respiratory chain acting as the major intracellular source of ROS [58–60]. An unavoidable respiratory electron leak from complexes I and III results in the formation of superoxide, O_2^- , which can react with lipids, protein and DNA [61–64]. The O_2^- can be readily converted into H_2O_2 either spontaneously or via a dismutation reaction involving manganese superoxide dismutase (MnSOD), a resident of the mitochondrial matrix. In the presence of redox active metal ions, H_2O_2 may, via the Fenton reaction, generate the highly reactive hydroxyl radical ($OH\cdot$) [56]. The $OH\cdot$ radical is responsible for multiple sites of mtDNA damage including single- and double-strand breaks, abasic sites and base modifications.

A further oxidative burden is caused by damage induced by O_2^- to Fe-S centers of mitochondrial proteins and includes subunits of complexes I, II and III as well as

aconitase [65–67]. Labile Fe-S enzymes such as mitochondrial aconitase, offer an important target for ROS. Of particular relevance to the eye, mitochondria located in cells exposed to visible light generate ROS via interactions with mitochondrial photosensitizers, like cytochrome c oxidase, to generate ROS and mtDNA damage [68, 69]. The transfer of energy from photoactivated chromophores to oxygen leads to the formation of singlet oxygen, $^1\text{O}_2$, which exists in an excited state. $^1\text{O}_2$ can generate ROS such as O_2^- by interacting with diatomic oxygen and by reacting directly with electrons with double bonds without the formation of free radical intermediates [70].

It is also important to note that specific tissues within the eye can also generate significant levels of ROS from nonmitochondrial sources. For example, lipofuscin (an age-related pigment that accumulates in RPE cells with age) is a potent photoinducible generator of ROS, and, in microvascular endothelial cells, NADPH oxidase is considered to be a major source of superoxide. Studies suggest that these ROS can also contribute to exogenous oxidative damage of the mitochondria, thus exacerbating mitochondrial dysfunction [69, 71, 72].

Mitochondrial Genome

Mitochondria are semiautonomous organelles that contain multiple copies of the 16,569-bp circular mtDNA. The mitochondrial genome encodes 37 genes, with 13 genes encoding protein subunits of the electron transport chain, and 24 genes encoding tRNAs and ribosomal RNAs needed for protein synthesis [73]. Moreover, a noncoding region referred to as the displacement loop or regulatory region is required for initiation of transcription and DNA synthesis. This noncoding region contains 2 hypervariable segments (HVS-I and HVS-II) with high polymorphism [74, 75]. The replication and repair of the mitochondrial genome are distinct from those of the nucleus and continue in postmitotic cells, such as the RPE and photoreceptors. The maintenance and repair of mtDNA are completely dependent upon proteins transcribed by the nuclear genome. mtDNA is organized into nucleoprotein complexes known as nucleoids that contain proteins directly bound to the DNA and which appear to associate mtDNA with the inner mitochondrial membrane [76]. It is generally assumed that the mtDNA is more sensitive to ROS and other chemicals compared to the nDNA [77].

mtDNA Damage and Repair

The stability of the mitochondrial genome is frequently challenged by endogenous and exogenous agents, including ROS which constitute the major endogenous source of mtDNA damage. mtDNA is particularly vulnerable to oxidative damage, due in part to its close proximity to the inner mitochondrial membrane where the majority of the ROS are generated and the fact that it does not contain histones which are thought to act as a physical barrier against ROS in the nuclear genome [6]. Furthermore it appears that oxidative damage to mtDNA is more extensive and persists longer compared to nDNA damage [77]. Such ROS-induced mtDNA damage includes base modifications, abasic sites, strand breaks and bulky adducts as well as a number of covalent modifications to DNA, which encompass single-nucleobase lesions, strand breaks, inter- and intrastrand cross-links, along with protein-DNA cross-links [78, 79]. The most studied oxidative lesion, 8-oxo-2'-deoxyguanosine, is capable of pairing with an adenine as well as cytosine during DNA replication, thus resulting in a G:C-to-T:A transversion mutation after 2 rounds of replication. Interestingly, levels of 8-oxo-2'-deoxyguanosine are greater in the mtDNA compared to the nDNA and are strongly correlated with large-scale mitochondrial deletions [80].

Mitochondria have less capacity for DNA repair than do nuclei [77, 81], and this is exacerbated by components of the mtDNA repair pathway being sensitive to oxidative inactivation [82]. Although the nucleotide excision repair pathway and recombination repair are absent in mitochondria, it is now known that mitochondria do indeed possess multiple alternative DNA repair pathways [6]. Oxidative mtDNA damage is thought to be repaired primarily by base excision repair [6, 83–86]. Double-strand break repair and mismatch repair have also been recently reported to be active in the mitochondria [87, 88]. DNA repair enzymes that are active against alkylated bases have also been identified within the mitochondrial compartment, and alkylated mtDNA is efficiently repaired in the mitochondria [6, 84, 89]. Thus, as the mtDNA molecule is highly susceptible to ROS-induced damage and mtDNA integrity is critical for cell survival, it is not surprising that multiple DNA repair pathways have evolved within the mitochondria [90]. The fact that each mitochondrion contains multiple copies of mtDNA also provides a significant measure of protection, given that heteroplasmic mutations do not lead to mitochondrial malfunction until they become the predominant species (see below).

Stochastic Mitochondrial Damage and Ocular Degeneration

Ocular tissues, including the retina, the optic nerve, photoreceptor cells and lens, exist in highly oxidizing microenvironments that are subjected to constant damaging light and/or UV wavelengths together with high oxygen fluxes, which strongly promote oxidative damage [4, 69, 91, 92]. Furthermore, environmental insults such as smoking add to the level of oxidative stress [93]. Research over the last few decades has provided compelling evidence of oxidative-stress-induced damage to mitochondria affecting protein, lipid and DNA and that resultant mitochondrial dysfunction contributes to the pathogenesis of several ocular disease(s) and in the aging process itself [4, 6, 92, 94] (fig. 1, 2). Because animal mitochondria are deficient in nucleotide and excision repair pathways [77], stochastic mtDNA defects can remain for life leading to the concept of 'metabolic memory' [95].

Age-Related Macular Degeneration

There is now strong support for mitochondrial genomic dysfunction being involved in AMD from clinical studies and animal models. There is a significant decrease in number and area of RPE mitochondria with increasing age, and these age-related changes are significantly increased in the eyes of AMD donors [96]. Recently, mtDNA haplogroups have been identified that are associated with either increased or decreased prevalence of age-related maculopathy [97–99]. These findings suggest that the bioenergetic consequences of mtDNA derangements may be expressed in macular RPE as a maculopathy or contribute to the development of AMD. A strong association between a variant of LOC387715/ARMS2 and AMD has been reported [100, 101]. Early reports suggested that ARMS2 is a mitochondrial protein, though these results have been questioned [102].

Evidence from a number of studies now strongly supports that mitochondrial dysfunction initiated by mtDNA damage is a feature that underlies the development of retinal aging and AMD. Increased mtDNA deletions have been documented in aged human and rodent retinas [103, 104] and increased mtDNA damage and decreased repair are associated with aging and AMD [104, 105]. Furthermore, the repair of the RPE mitochondrial genome appears to be slow and relatively inefficient [106]. Photoreceptor outer segments have been shown to damage RPE mtDNA in vitro, by a burst of ROS generated during ingestion. Blue light exposure

also harms mitochondrial respiratory activity and damages mtDNA [69]. In addition, a recent report shows that the aged RPE and choroid of rodents suffer extensive mtDNA damage and that this is likely to be due to decreased DNA repair capability [104]. Changes in selected redox proteins and proteins involved in mitochondrial trafficking [107–109] and a decrease in RPE mitochondrial respiration also correlate with AMD progression [105]. Knockdown of MnSOD, the mitochondrial antioxidant responsible for neutralizing superoxide anions, results in pathological lesions in mice similar to those observed in 'dry' AMD [110]. Ex vivo studies show that RPE cells exposed to high levels of ROS suffer preferential damage to mtDNA and subsequent repair is poor [89, 111, 112].

Uveitis

Intraocular inflammation, commonly referred to as uveitis, is a principal causative factor underlying blindness from retinal photoreceptor degeneration. Oxidative retinal damage in uveitis is caused by activated macrophages, which generate various cytotoxic agents, including inducible nitric oxide produced by inducible nitric oxide synthase, O_2^- and other ROS [113]. Oxidative stress plays an important role in the photoreceptor mitochondria during the early phase of experimental autoimmune uveitis (EAU). It has been shown that mtDNA damage occurs early in EAU; interestingly, nDNA damage occurred later in EAU [5]. Furthermore, mitochondrial proteins in the photoreceptor inner segments are modified by peroxynitrite-mediated nitration [114] which, in turn, leads to increased generation of mitochondrial ROS. In support of an increased state of mitochondrial oxidative stress, MnSOD has been shown to be upregulated during EAU, presumably to counteract ROS [115]. Recent data appear to suggest a causative role of oxidative mtDNA damage in the early phase of EAU, before leukocyte infiltration. Such oxidative damage in the mitochondria may be the initial event leading to retinal degeneration in uveitis [5].

Glaucoma

Increasingly persuasive evidence suggests that glaucomatous tissue damage initiated by elevated intraocular pressure and/or tissue hypoxia also involves oxidative stress. Experimental elevation of intraocular pressure induces oxidative stress in the retina. It also appears that mitochondrial oxidative stress may have an important role in glaucomatous neurodegeneration [116–118]. In glaucoma there is evidence that mitochondrial dysfunction

tion may lower the bioenergetic state of retinal ganglion cells leading to an increased susceptibility to oxidative stress and apoptotic cell death [118, 119]. In addition, light exposure may be an oxidative risk factor, whereby it reduces mitochondrial function and increases ROS production in ganglion cells [120]. Mitochondrial dysfunction has also been implicated in neuronal apoptosis in experimental models of glaucoma [121, 122]. mtDNA abnormalities further support a role of mitochondrial-dysfunction-associated stress as a risk factor for glaucoma patients [123].

Diabetic Retinopathy

Mitochondrial dysfunction has been shown to play an important role in diabetic retinopathy [94, 118]. Retinal mitochondria experience increased oxidative stress in diabetes, and complex III is one of the major sources of the increased O_2^- [124]. Superoxide levels are elevated in the retina of diabetic rats and in retinal vascular endothelial cells incubated in high-glucose media [125], and hydrogen peroxide content is increased in the retina of diabetic rats [126]. Membrane lipid peroxidation and oxidative damage to DNA, the consequences of ROS-induced injury, are elevated in the retina in diabetes [127]. Chronic overproduction of ROS in the retina results in aberrant mitochondrial functions in diabetes [94], and hyperglycemia-induced overproduction of superoxide by the mitochondrial electron transport chain is considered to activate the major pathways of hyperglycemic damage by inhibiting GAPDH activity. However, the mechanism by which hyperglycemia causes an increase in mitochondrial ROS is not yet fully understood, with some implicating a direct effect and others an indirect role via high-glucose-induced cytokines [128–131]. Elevated O_2^- levels activate caspase 3 which leads to cell death in the retinal capillaries [94]. Upregulation of SOD2 inhibited diabetes-induced increases in mitochondrial O_2^- , restored mitochondrial function and prevented vascular pathology both in vitro and in vivo [124, 132–134]. However, timing of such treatments is critical since animal studies have demonstrated that oxidative stress contributes not only to the development of diabetic retinopathy, but also to the resistance of retinopathy to reversal after good glycemic control has been re-instituted – the metabolic memory phenomenon [135]. Resistance of diabetic retinopathy to reversal is possibly attributable to accumulation of damaged molecules in mitochondria and ROS-induced damage that is not easily removed even after good glycemic control has been reestablished. However, the accumulation of advanced

glycation end products is also implicated in metabolic memory [136].

Variation in mtDNA has also been linked to resistance to type 1 diabetes. A single nucleotide change (C5173A), resulting in a leucine-to-methionine amino acid substitution in the mitochondrially encoded NADH dehydrogenase subunit 2 gene, is associated with resistance to type 1 diabetes in a Japanese population [137]. Similarly, an orthologous polymorphism (C4738A), resulting in an L-to-M substitution, provides resistance against the development of spontaneous diabetes compared with the diabetes-prone nonobese diabetic mouse strain [138]. Gusdon et al. [139] have shown that the methionine substitution results in a lower level of ROS production from complex III.

Cataract

Oxidative stress plays a significant role in cataractogenesis [92, 140]. The lens is highly susceptible to ROS, and mitochondria are located in the epithelium and superficial fiber cells. Interestingly, in these cell types, the mitochondria have been confirmed as the major source of ROS generation [141]. Several in vitro studies have demonstrated that human lens cells are highly susceptible to oxidative insults, in which antioxidant activity is generally inversely proportional to cataract severity [142]. Oxidation of proteins, lipids and DNA has been observed in cataractous lenses [143–145]. Proteins from cataractous lenses lose sulfhydryl groups, contain oxidized residues, generate high-molecular-weight aggregates and become insoluble [140]. In addition, cataract has been shown to be a symptom of a newly identified mitochondrial disease called autosomal-recessive myopathy, caused by mutations in the growth factor, augmentin of liver regeneration gene, which affects protein levels of the mitochondrial intermembrane space [146].

Conclusion

The findings discussed highlight the importance of mtDNA damage arising from both stochastic ROS and maternally inherited mutations. mtDNA genomic instability is a principal defect in the etiology of multiple diseases that result in ocular manifestations (summarized in fig. 2). Continued research in this clinically relevant area will undoubtedly provide a greater understanding of how deficiencies/failure of the mitochondrial genome contribute to the pathogenesis of degenerative diseases. Future challenges will involve developing therapeutic strategies

that target mitochondria and manipulate mtDNA replication, repair and levels of nucleotide precursors to maintain mtDNA integrity with the ultimate goal of blocking or retarding the effects of mitochondrial disease.

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