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DNA damage and repair in age-related macular degeneration

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1. ABSTRACT

Oxidative stress may play an important role in the pathogenesis of age-related macular degeneration (AMD). Mitochondria produce reactive oxygen species (ROS), which induce degenerative changes typical for AMD. Mitochondrial DNA (mtDNA) is targeted by ROS and it is considered to be more vulnerable to damage than nuclear DNA (nDNA) due to the impaired DNA repair system, lack of nucleosomal organization and close vicinity of mitochondrial oxidative chain. Some reports suggest the association between mtDNA damage and AMD. However, the metabolism of mtDNA is mainly determined by the expression of nDNA. Therefore, the extent of damage to mtDNA in retinal cells depends on the overall efficacy of nDNA repair, which decreases with age. We showed an association between nDNA damage and repair and AMD. Also well-recognized factors of AMD pathogenesis, age and smoking, may exert their effects through the DNA damage and repair. In conclusion, DNA damage and repair, both in mitochondrial and nuclear genome, may play an important role in the pathogenesis of AMD, and their mutual relationship in this disease needs further study.

2. INTRODUCTION

Age-related macular degeneration (AMD) belongs to the most common and serious ocular diseases, but despite its importance and extensive research, its pathogenesis has not been completely elucidated. This is important, because currently there is no cure for the disease and treatment options are limited. In general, AMD is seen as a complex disease with several risk factors, including age, gender, tobacco smoking, race and diet (1). A growing body of evidence suggests that reactive oxygen species (ROS) may directly damage retinal pigment epithelial (RPE) cells, contributing to retinal degeneration, a clinical hallmark of AMD (2). However, the source of these species is not conclusively recognized. They can be produced during the oxidative stress associated with smoking or sunlight exposure and their action may be potentiated by disturbed iron metabolism (3). ROS may damage virtually all cellular components – proteins, lipids and DNA/RNA molecules – and oxidative damage is believed to be involved in the pathogenesis of many serious diseases and aging. Therefore, searching for the association between reaction of cell to DNA damage may help to recognize mechanisms underlying some diseases.

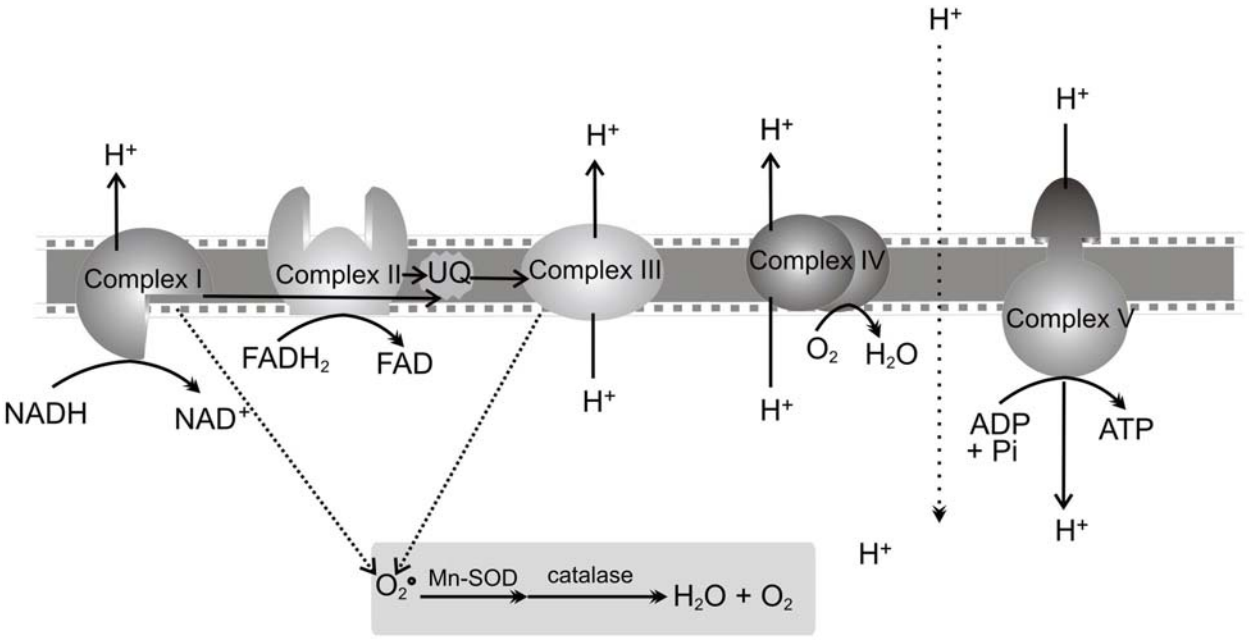

Figure 1. Schematic representation of the mitochondrial electron transport complexes I to V. Electrons flow from reducing equivalents NADH or $FADH_2$ to complex I or II, respectively and then to ubiquinone (UQ) pool. Then electrons flow through complexes III and IV to the final acceptor, molecular oxygen. ADP is phosphorylated to ATP at complex V (ATP synthase) due to the proton gradient kept along with the movement of electrons. Single electrons interact with molecular oxygen at complexes I and III to generate the superoxide radical ($O_2^{\bullet}$). The subsequent actions of superoxide dismutase (SOD) and catalase change superoxide into oxygen and water.

Mitochondrial DNA (mtDNA) is often considered as a more vulnerable to damage, especially to oxidative stress, than nuclear DNA (nDNA). There are several reasons for such difference, including immediate vicinity of mitochondrial respiratory complexes generating ROS, impaired DNA repair, lack of nucleosomal organization, constant division during mitochondrial biogenesis, lack of intervening introns in mitochondrial genes. However, the metabolism of mtDNA is regulated mainly by the products of the expression of nuclear genome, so the damage to nDNA may determine the extent of damage observed in mtDNA.

3. MITOCHONDRIAL GENOME AND PHYSIOLOGY

Human cells not only have a nuclear genome but also cytoplasmic genomes, which are compartmentalized in the mitochondria. The human mitochondrial genome has been sequenced well before the nuclear genome. It is a circular, negatively supercoiled double stranded 16,569 bp long DNA molecule and contains 37 genes encoding proteins of respiratory complexes I, III and IV, ATP synthase, rRNAs and tRNAs (4). Apart from a small fragment called D-loop, the mtDNA genome contains contiguous or overlapping genes with no non-coding intervening sequences or such sequences are limited to a few bases. The genetic code in mtDNA differs slightly from the nuclear genome, resulting, in some instances, in the lack of genes encoding the termination codons (5). The two strands of mtDNA are asymmetric in base composition and are called light and heavy. The human mtDNA is of maternal origin and mitochondria do not have histones, but the human mtDNA is organized in nucleoids containing several mtDNA-binding proteins (6). Maintenance and transcription of mtDNA is dependent on the nuclear-encoded proteins. mtDNA replicates for entire life of an organism so that mitochondria can undergo continuous turnover (7, 8). Due to asymmetry of mtDNA strands, there are bidirectional models of their replication (9), led by DNA polymerase gamma, but these models have been challenged by reports suggesting a normal leading/lagging strands synthesis (10).

Mitochondria are essential organelles in all eukaryotic cells. They regulate many important processes including redox reactions, calcium homeostasis and synthesis of several biomolecules. Their main role is the production of cellular energetic resources in the form of ATP molecules, as they serve as a major source of ATP. The inner membrane of the mitochondrion maintains a high electrochemical gradient, in which many transporters and enzymes function.

Mitochondrial oxidative phosphorylation converses macronutrient energy to ATP in a set of reactions in which nutrients, such as fatty acids, glucose and amino acids, are oxidized, oxygen is reduced to water and ADP is phosphorylated to ATP (Figure 1). The process is initiated by entering the tricaboxylic acid cycle by carbon substrates, which are then oxidized, producing reducing equivalent in the form of NADH, and $FADH_2$ inducing and electron flow

through respiratory chain complexes I (NAD dehydrogenase) and II (succinate dehydrogenase), respectively. The electron flow through complexes I and II merges on complex III (ubiquinone-cytochrome *c* reductase) with electrons from electron transferring flavoproteins. This is beta oxidation with the contribution of the mobile electron carrier coenzyme Q. Another such carrier transfers electrons onto complex IV (cytochrome *c* oxidase), where they are transferred to oxygen, producing water. This electron transport through complexes I, III and IV generates a proton gradient across the inner mitochondrial membrane, which fuels phosphorylation reaction in complex V (ATP synthase) converting ADP into ATP.

One of the consequences of the reactions in the respiratory chain is the production of reactive oxygen species, which can damage cellular structures. In fact, mitochondria are mainly responsible for the majority of cellular ROS, although cyclooxygenases, NADPH oxidase, and peroxisomes cannot be overlooked. Mitochondria have their own system against ROS. Superoxide is converted to hydrogen peroxide (H_2O_2) by the action of superoxide dismutase, which can be in two forms: manganese superoxide dismutase (SOD1) in the mitochondrial matrix and copper-zinc superoxide dismutase (SOD2) in the cytosol. Hydrogen peroxide is deactivated by catalase, glutathione peroxidase or peroxiredoxins (11).

4. MITOCHONDRIAL MUTAGENESIS

The rate of nucleotide sequence divergence for mtDNA is about 10-fold more rapid than for nDNA (12). The reason for this difference does not lay in differences in the efficacy of DNA repair or accuracy of DNA polymerases synthesizing DNA in replication. Many mutations in mtDNA have been associated with serious diseases (12, 13). The mitochondrial genome may be damaged in the same way as the nuclear genome and the only difference may lay in possible phenotypic consequences of such damage, because damage to the mitochondrial genome becomes apparent only when a large number of mtDNA molecules are damaged at the same time. However, several factors distinguish mtDNA from nDNA, which can underline its susceptibility to the DNA-damaging factors. These are the lack of nucleosomal organization, the proximity to the inner mitochondrial membrane, which is a rich source of ROS and enhanced susceptibility to certain lipid-soluble polar chemicals. In consequence, there are high steady levels of oxidative DNA damage in mtDNA (14).

Because there is a negative charge on the matrix-side of the inner mitochondrial membrane, lipophilic cations tend to accumulate in mitochondria, specifically in mitochondrial membranes. Mitochondria are easily penetrated by lipophilic cations from the cytosol, which are then concentrated in the organelle up to 1000-fold (15). Therefore, many chemicals, including drugs, which are lipophilic and have positive charges, are concentrated in mitochondria, constituting a hazard for mitochondrial structures, e.g. mtDNA is alkylated at the extent of about 10-fold more than its nuclear counterpart (16).

Mitochondrial genetics is complex due to the presence of many copies of the mitochondrial genome in individual mitochondria. The presence of both mutated and wild-type mtDNA in the same cell or tissue is termed heteroplasmy (17). In the presence of heteroplasmy, the mutated mtDNA is functionally recessive, and a biochemical defect is present only if there is more than 60% to 65% mutated mtDNA within an individual cell (18, 19). Therefore, the presence of low relative number of mutated mtDNA copies may not affect cell functions.

The most effective and versatile DNA repair pathway, nucleotide excision repair (NER), has not been identified in mtDNA, and in the case of bulky DNA damages requiring NER, mtDNA undergoes degradation (20). Base excision repair (BER) was firstly shown to operate in mammalian mtDNA (21). Despite that some mismatch repair (MMR) enzymes are located in the mitochondrion, operation of this DNA repair mechanism in mtDNA has not been reported. There is a growing body of evidence for the presence of direct reversal of base damage in mtDNA (22, 23).

The only well documented mode of BER in mtDNA is short-patch BER (24). The repair of oxidative mtDNA damage was shown to be universal, fast and effective (25). Enzymology of BER in mitochondria is simple compared to its nuclear counterpart and is based on a few glycosylases, an apurinic-apyrimidinic nuclease, DNA polymerase gamma, DNA ligase and an endonuclease which can be involved in alternative excision repair (24). Basic mechanism of BER in mtDNA is very similar to that operating in the nucleus. Briefly, an AP site generated by the action of a glycosylase is cleaved by an AP endonuclease, generating 5' incision with 3'-OH and 5'-deoxyribosephosphoryl termini. The 5' termini are removed by a specific lyase activity, which enables the synthesis by polymerase gamma and ligation. Therefore, in contrary to nucleus, only short-patch BER operates in mitochondria. Recombination may take place between any mtDNA molecules, which rises a question on operating of homologous recombination repair in the mitochondrion (26, 27). There are several strong evidences coming from other than human organisms on the involvement of such repair in the removing of oxidative DNA damage (28, 29). It is important to note that results on DNA repair in mtDNA in lower eukaryotes cannot be directly extrapolated to higher organisms.

Several reports suggest age-related slow-down in the efficacy of DNA repair and DNA damage accumulation (30-32). This is in line with the DNA theory of aging formulated by Alexander in 1967 (33). According to this theory, the accumulation of DNA damage leads to the deregulation of cellular function and thereby to aging. Nowadays we realize that the cellular response to DNA damage includes a variety of actions, including DNA repair, inhibition of replication and transcription, changes in cell cycle, apoptosis, senescence, necrosis and other

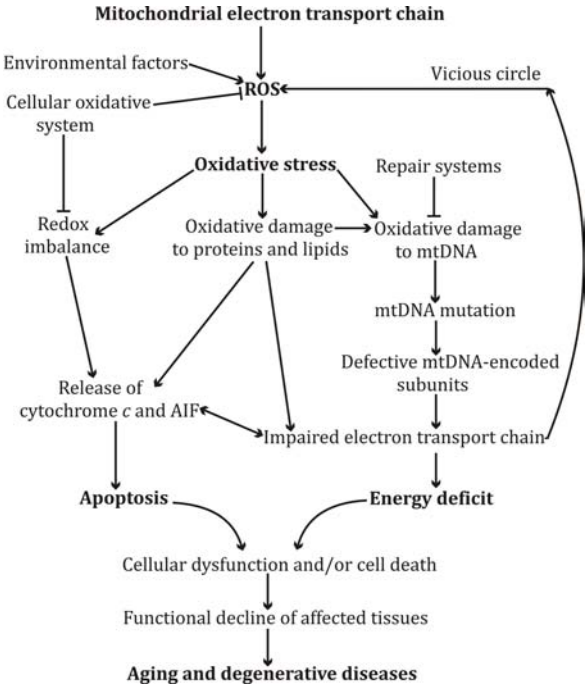

Figure 2. "Vicious cycle" leading from mitochondrial electron transport chain to aging and degenerative diseases. Reactive oxygen species (ROS), produced in the electron chain, damage cellular biomolecules, which interfere with the structure and functions of the chain, resulting in increased ROS production. This cycle is propagated in an age-dependent manner (adapted from (103)).

processes (34-36). Deleterious genetic changes may gradually accumulate due to oxidative DNA damage (37). This suggests the accumulation of certain poorly repaired forms of DNA damage with age (38). However, the fundamental question whether accumulation of DNA damage and impaired DNA repair is the reason or a consequence of aging is still open. Therefore, research exploring the association of impaired DNA repair and accumulation of DNA damage and age-related syndromes, including AMD, may help to answer this question. If the accumulation of oxidative DNA may contribute to aging and age-related diseases, then mtDNA may be considered as a target of such research. The role of mitochondria in the aging process has been a topic of intense interest for many years. So-called mitochondrial theory of aging postulates that the accumulation of oxidative DNA damage in the mitochondrial genome results in a progressive weakening of the cellular function, eventually leading to aging process and age-related diseases (39, 40) (Figure 2).

The mitochondrial inner membrane is a site for the electron transport system involved in ATP synthesis by respiration, consuming about 90% of oxygen taken by the cell. A part of the oxygen is incompletely reduced generating reactive oxygen species, which are targets of the cellular antioxidant protection system, a system containing mainly antioxidant enzymes and small molecules of antioxidants. Mitochondrial oxidative phosphorylation is a potent source of ROS with up to 5% of the oxygen picking up electrons directly from the flavin dehydrogenases and ubiquinol to generate superoxide radicals (14). Some ROS are not effectively processed by the system and can interact with nearby mtDNA evoking oxidative DNA damages, which can be turned into mutations. Mitochondrial oxidative capacity decreases by about 8% per decade using substrates providing electron flow into complex I, complex II, and electron-transferring flavoprotein (41). Moreover, mtDNA copy number decreases with age (41-43), which could imply the reduction of mitochondrial gene transcripts and therefore, the proteins encoded by these genes. There are reports suggesting that the effects of aging on mtDNA abundance and gene expression may be translated to alterations and mutations to portions of the nDNA that encode mitochondrial proteins (44). On transcription and translation, damaged mtDNA gives defective subunits of electron transport chain, which produces more and more ROS, affecting various molecules in mitochondria. This is a vicious circle leading to accumulation of mutations and faulty biomolecules, of which activity sponsors bioenergetic decline of the cells in the aging process. Concurrently, the efficacy of the cellular antioxidative system is decreasing. Additionally, increased levels of ROS may induce apoptosis by depleting reduced glutathione and ATP, changing cellular redox potential, reduction of NADH, NADPH and other reducing agents. These changes may sponsor lipid peroxidation, and these products can damage mtDNA and change the structure of mitochondrial membrane resulting in leaking of cytochrome *c* and apoptosis inducing factor (AIF). Therefore, apoptosis pathways may be activated as a consequence of mitochondrial age-related overproduction of ROS. These all cell-devastating processes act in concert producing decline of physiological and biochemical functions of the cell and the tissue.

5. MITOCHONDRIAL DYSFUNCTION IN AMD

Mitochondria play a central in the pathogenesis of age-related degenerative diseases (45). Several studies reported some defects in mtDNA and consequent perturbations in the function of respiratory enzyme complexes associated with diminished energy production, increased generation of ROS and induction of apoptosis (46). Aging is associated with perturbations in macular function, with progressive decrease in both visual acuity and contrast sensitivity. This process is accompanied by some morphological changes in the photoreceptors and RPE cells – atrophy and loss of both kinds of cells, hyperpigmentation and depigmentation of RPE cells, progressive accumulation of lipofuscin, formation of the drusen, thickening of Bruch's membrane, and basal laminar deposits (47). In some individuals these changes are expressed to the form typical for AMD.

The retina is composed of highly metabolically active postmitotic cells and as a consequence, particularly sensitive to mutations in mtDNA (48). Additionally, the retina is exposed to light, including blue and ultraviolet (UV) radiation, causing damage to DNA, including mtDNA (49-53). The accumulation of mutations in mtDNA in aging macular photoreceptors has been reported (54).

These mutations were predominantly present in the photoreceptor layer of the retina, which may suggest that exposure to blue and UV light may play a role in such accumulation. The amount of deleted mtDNA in the retina cells increased with age, as in other tissues (55, 56). A significantly higher level of mtDNA mutations was observed in the photoreceptor layer than in other ageing tissues (57). As mentioned in the previous section, the number of mutated mitochondrial genomes in a cell must be relatively high to affect its function. However, individual deletions in ageing mtDNA may be clonally expanded to exceed the sufficient number to cause biochemical defects (57, 58). In general, low levels of mtDNA4977 mutation in aging retina were observed (17, 18, 59, 60). In the study previously mentioned, cytochrome oxidase-negative photoreceptors were reported in the central retinas, the region where the highest level of mutated mtDNA was observed (54).

The accumulation of mitochondrial damage sufficient enough to induce a biochemical defect may have several phenotypic consequences to a cell. Such a cell may display a decreased oxidative capacity with profound implications on ATP-dependent cellular functions, including photoreceptor-specific ATP-binding cassette transporter, which appears to play a crucial role in the cycling of retinoids between the RPE and neural retina (61, 62). The inhibition of this transporter activity may cause, typical for AMD, accumulation of the lipofuscin-like material in the RPE cells (62, 63). The accumulation of mtDNA damage may also play a role in the induction of apoptosis and, consequently, loss of photoreceptors, typical for the aging retina (64, 65).

Mitochondrial alterations in degenerative diseases may also involve some structural and functional changes to mitochondrial membrane, including lipid transport and metabolism, cholesterol biosynthesis, activity of pyruvate dehydrogenase complex and apoptosis (66-68). These changes may impair electron transport chain, energy production and overall homeostasis of the cell. The alterations in the mitochondrial membrane were reported also in AMD (71). A progressive deterioration of the membrane was observed with aging, occurring in association with peroxisome proliferation and accumulation of lipofuscin in RPE cells in that study. Moreover, alterations of the RPE mitochondrial membrane and proliferation of peroxisomes were more pronounced in AMD than in normal aging (69, 70). This data suggests that the loss of mitochondrial structure is one of the main differences between normal and AMD-affected RPE cells (69, 70).

Loss of mitochondrial functions in RPE cells may imply changes in the expression of nuclear genes, which may have serious consequences for the functioning of these cells and the retina (72). The mechanisms underlying the association of AMD with mitochondrial dysfunction may involve altered mitochondrial translation, import of nuclear-encoded proteins and ATP synthase activity (73). These processes are regulated by the expression of specific mitochondrial proteins. A global proteomics analysis revealed change in several mitochondrial proteins in RPE isolated from individuals with AMD (74).

It was shown that the induction of mitochondria-derived ROS may play a critical role in the death of cells exposed to short-wavelength blue light (about 425 nm) (75). ROS and cell death are blocked either by inhibiting the mitochondrial electron transport chain or by mitochondria-specific antioxidants. These results show that mitochondria are an important source of toxic oxygen radicals in blue light-exposed RPE cells and may indicate new approaches for treating AMD using mitochondria-targeted antioxidants.

Due to the critical role of mitochondria in supplying energy to the cell, quality control of these organelles is important to maintain cellular homeostasis. It has been assumed that autophagy (mitophagy) is the pathway for mitochondrial recycling, and that the selective degradation of mitochondria via mitophagy is the primary mechanism for mitochondrial quality control. Recent studies identified several mitophagy-related genes and have revealed components involved in the molecular mechanism and regulation of mitophagy (76). On the other hand autophagy may play an important role in AMD, so mitophagy may be of a special significance in this disease (77, 78).

In summary, reduction of mitochondrial function, which may be a consequence of the loss of mitochondria, may be associated with the development of AMD. Although it is tempting to establish mitochondrial dysfunctions as a causative factor in the pathogenesis of AMD, in fact we do not currently know whether mitochondrial changes are cause or consequence of AMD. Both possibilities cannot be excluded, as in the case of the relationship between mtDNA damage and alterations in the mitochondrial structure.

6. MITOCHONDRIAL DNA DAMAGE AND REPAIR IN THE RETINA AND RPE CELLS

It was shown that human mtDNA of RPE cells in culture is more susceptible to the damage to oxidizing or alkylating agents than its nuclear counterpart in these cells (79-81). The action of any DNA-damaging agent onto cellular (mitochondrial or nuclear) DNA depends on the ability of the cell to manage with the consequences of such action. This ability is, in turn, determined by the capacity of the cell to decrease the DNA-damaging potential of the compound and DNA repair efficacy. Data presented in the previous sections suggest that in general DNA repair is less efficient in mtDNA than in nDNA. This concerns also RPE cells (82). However, it was suggested that RPE cells might have higher efficacy to repair nDNA than other cell types to survive permanent oxidative stress they had to cope with (80). Moreover, RPE cells may be characterized by the enhanced capacity to scavenge ROS by antioxidative enzymes and small antioxidants in the response to the oxidative stress (83). Such an increase in the capacity to withstand with enhanced oxidative stress suggest that this reaction had adaptative character. However, mtDNA does

not seem to have such extra protection and this may suggest that the mitochondrion can be a "a weak link" in the RPE cells' system of fighting with oxidative stress (84). Moreover, the RPE cells lack active poly(ADP-ribose) polymerase (PARP), which significantly lowers their DNA repair capacity (81). Because the mitochondria are rich source of proteins regulating apoptosis, accumulation of mtDNA damage may result in the activation of a mitochondrial apoptotic pathway. Moreover, the elevated expression of Bcl-2, an anti-apoptotic protein, may accelerate the repair of mtDNA in RPE cells (85). In general, we speculate, that the increased level of mtDNA damage in RPE cells may be a consequence of impaired DNA repair in these cells. This hypothesis has been confirmed by the results of research showing preferential damage to mtDNA in choroid and RPE cells, its accumulation with age as well as the decreased expression of some DNA repair enzymes (86).

The increased DNA damage to mtDNA and its decreased repair was shown to correlate with stages of AMD and a decrease in the respiratory chain activity in RPE cells (87).

These data strongly suggest the involvement of mtDNA damage and repair in the pathogenesis of AMD. However, the contribution of disturbances in mtDNA metabolism to AMD pathogenesis may be extended onto overall genomic instability, because there is strong dependence of the metabolism of mtDNA on the stability of nDNA.

7. ARE DNA DAMAGE AND REPAIR IN THE NUCLEUS INVOLVED IN AMD?

As discussed above, mitochondrial DNA may be involved in the induction and progression of AMD. Therefore, an immediate question arises about possible involvement of nDNA in AMD. There is no doubt that nDNA is better protected from endogenously generated ROS than mtDNA. But one of the main arguments for the significance of mtDNA mutagenesis in AMD is relatively poor efficacy of the repair of mtDNA and its decrease with age. The latter effect concerns also nDNA and it may result in more pronounced consequences of the cell than changes in mtDNA (88). However, it was shown that retinal mtDNA had greater numbers of rearrangements/deletions compared to blood mtDNA in normal samples and AMD samples (89). Therefore, individuals with AMD may be characterized by high levels of large mtDNA deletions/rearrangements in the retinas, changes in the nucleotide sequence, which are more pronounced in the coding than non-coding region of mtDNA. These changes may be involved in the diminishing energy production and disturbed sending of genetic information and its expression in mtDNA. However, because regulation of mtDNA metabolism, including its repair, is regulated mainly by proteins encoded in the nucleus, instability of the nuclear genome contributes to the instability of its mitochondrial counterparts.

Our team has focused on the role of damage to nDNA in AMD. The issue of the coupling of mtDNA with nDNA is clear, but some general features of the DNA molecules associated with the aging process may also be important in the pathogenesis of AMD. Several environmental factors can be involved in the pathogenesis of AMD, including UV radiation and blue light exposure, although the issue of the significance of phototoxicity in AMD is still the matter of debate (90). The possible involvement of light exposure in the pathogenesis of AMD is partly explained by the accumulation of lipofuscin in aging RPE cells and increasing the phototoxicity risk of the retinal cells in elderly (91). However, several major epidemiological studies did not correlate AMD and the exposure to light, but such studies encounter many obstacles, including difficulty in the estimation of the magnitude of the exposure and in the determining of "genetic background", influencing the interaction with light (92).

Although the contribution of light exposure to the induction and/or progression of AMD is not proven unequivocally, there is no doubt that the light may damage the retina by mechanism, which can be broadly divided into three classes: photochemical, photothermal and photomechanical (93). Photochemical injuries to the retina can be, in turn, classified into several symptoms depended on the light wavelength. There is a positive correlation between the severity of one of such symptoms, UV-blue photic retinopathy ("blue light hazard") and lipofuscin accumulation (94). Therefore, the role of UV radiation and blue light can be considered in the context of the role of mtDNA in the pathogenesis of AMD, because both radiations can damage DNA. We think that the problem of contribution of the interaction of mitochondrial and nDNA with UV radiation and blue light is complex, but certainly such interaction is possible in the photoreceptor cells. We have reasoned in the following way. In general, the efficacy of DNA repair decreases with age, so we can consider an average level of DNA repair at a particular age. This average is a result of both higher and lower individual repair capacities, determined among different members of a population. If an individual has a lower efficiency of DNA repair than average for his/her age, he/she would be more susceptible to the DNA-damaging factors, including UV radiation and blue light. This concerns both nuclear and mitochondrial DNA. In other words, the constitutive efficacy of DNA repair, typical for a given age, may contribute to the pathogenesis of AMD.

In our previous work we showed that individuals with AMD displayed a higher extent of basal endogenous DNA damage without differences between patients of dry and wet forms of the disease (95). DNA double-strand breaks did not contribute to this extra DNA damage. The extent of oxidative modification to DNA bases was grater in AMD patients than in the controls. Lymphocytes from AMD patients displayed a higher sensitivity to hydrogen peroxide and UV radiation and repaired lesions induced by these factors less effectively than the cells from the control individuals. Therefore, an impaired efficacy of DNA repair may combine with enhanced sensitivity of the retina and

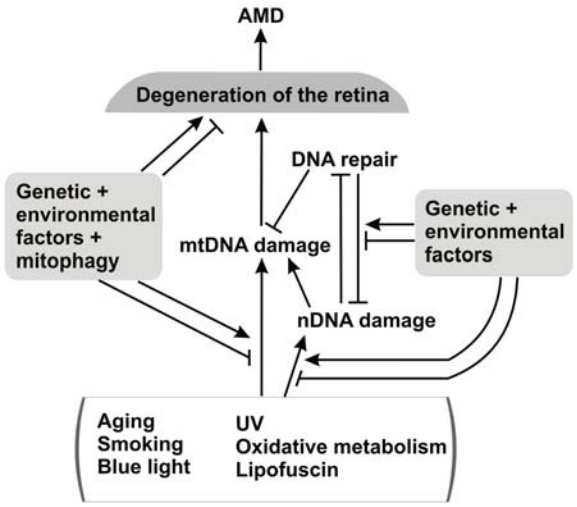

Figure 3. Interactions between mitochondrial and nuclear DNA (mtDNA and nDNA, respectively) modulated by environmental and genetic factors leading to degeneration of the retina and age-related molecular degeneration (AMD).

RPE cells to blue and UV lights, contributing to the pathogenesis of AMD. We obtained similar results in our other study (96). Additionally, we did not find any correlation between the lowered efficacy of DNA repair and the genotypes of the Ser326Cys polymorphism of the *h*OGG1 gene, which product, the hOGG1 protein, is a DNA glycosylase removing oxidative lesions from DNA, including 8-hydroxy guanine. Because the protein acts in base excision repair, variability in the genes of other DNA repair pathways may be associated with the differences between DNA repair in AMD patients and normal subjects. In the next work we showed, that polymorphism of another gene, which product removes oxidative DNA damage, *MUTYH*, may not be directly associated with AMD occurrence and progression, but may modulate the influence of industrial or rural environment as a risk factor in this disease (97).

8. PERSPECTIVE

Mitochondrial DNA is considered as more vulnerable to oxidative damage than nDNA for several reasons, including direct contact with the reactive oxygen species produced in the mitochondria, the lack of nucleosomal organization and lesser efficacy of DNA repair. However, the latter is mainly determined by the expression of nDNA, which is one of many examples illustrating the dependence of mtDNA from its nuclear counterpart. Therefore, if we consider the involvement of mtDNA in AMD pathogenesis, we should take into account, that this can be partly underlined by the changes in the nuclear genome. The results we obtained suggest such possibility. The role of phototoxicity in the pathogenesis of AMD, which is not firmly establish, can be explained by the interaction of UV radiation and blue light with DNA and the accumulation of lipofuscin under the influence of these radiations. The role of phototoxicity in the pathogenesis of AMD, which is not firmly established, can be explained by the interaction of UV radiation and blue light with DNA and the accumulation of lipofuscin under the influence of these radiations. Lipofuscin may be a source of ROS, which can damage DNA, but it was reported to damage rather nuclear than mitochondrial DNA (98). However A2E, a weak phototoxic pyridinium bisretinoid component of lipofuscin, can be found in mitochondria and may produce additional DNA damage (99).

Aging and smoking are well established risk factors in AMD. However, both these factors may also influence mitochondrial function (100, 101). Therefore, mitochondrial dysfunctions observed in AMD may result partly from aging and smoking. However, as we discussed above, aging results in the decrease in DNA repair and tobacco smoke contains many DNA-damaging agents (102).

In summary, we conclude that mtDNA damage and repair may be directly involved in the pathogenesis of AMD, but this involvement is considerably influenced by these processes in nDNA. However, mtDNA damage of the target cells, first of all the retina and RPE cells, seems to play a major role in AMD pathogenesis, while damage to nDNA may potentiate damage to mtDNA in these cells. The interrelationships between mitochondrial and nDNA damage and repair and other factors contributing to the pathogenesis of AMD is displayed in Figure 3 The implications of damage and repair in nDNA for AMD warrants further studies.

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