Review

Oxidative DNA Damage and Proteostasis in Age-Related Macular Degeneration

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\textbf{Abstract:} Age-related macular degeneration (AMD) is a severe eye disease the prevalence of which continues to rise along with the increase in living expectancies. Both genetic and environmental factors contribute to the onset and progression of the disease in which the capacity of retinal pigment epithelium (RPE) cells to support the photoreceptors is diminished. RPE cells are heavily exposed to oxidative stress which predisposes body’s metabolically most active cells to several threats. Protein homeostasis or proteostasis stands on the mTOR interconnecting pathways that are involved in controlling the production, folding, transporting, posttranslational modification, and degradation of proteins. We will provide here a view how oxidative stress and the subsequent DNA damage result in disturbances in the proteostasis of aged cells and thereby contribute to the development of aggregation diseases, such as AMD.

\textbf{Keywords:} DNA damage, macular degeneration, oxidative stress, proteostasis, retinal pigment epithelium

1. Introduction

Age-related macular degeneration (AMD) is a progressive eye disease of the central part of the retina leading to a vision loss in the elderly. AMD is a multifactorial disease with many genetic and environmental factors, most of which are associated with the generation of oxidative stress and chronic inflammation (Fig.1) [1]. Decline in the vision quality is a result of the degeneration and atrophy of retinal pigment epithelial (RPE) cells and neural cells (rod and cones). Since it is the responsibility of RPE cells to maintain the functionality of photoreceptors, the overlying rod and cone cells become deprived due to an irreversible damage on RPE cells. AMD is usually categorized into two main classes: dry (atrophic) form and wet (exudative) form that account for about 85% and 15% of AMD, respectively (Fig. 2) [2]. Both AMD classes show RPE hyper- and hypo-pigmentation, formation of lipofuscin, the presence of drusen and cell loss, whereas only for wet AMD, rapid and sudden loss of vision due to subretinal neovascularization (between the retina and choroid) is observed (Fig. 2). Currently, there is no proven treatment for dry AMD, whereas the activity of wet AMD may be inhibited with the intraocular injections of antiangiogenic agents [3]. Lipofuscin and drusen accumulations reflect to severity level of AMD [4]. They are indication of disturbed proteostasis in conjunction with chronic oxidative stress, DNA damage, mechanistic target of rapamycin (mTOR) signaling and inflammation [1, 4].

2. Oxidative stress and AMD

The highest consumption of oxygen per cell in humans is in the retina. Oxygen is a potent source of reactive oxygen species (ROS), including both oxygen radicals ($\text{O}^\cdot_2$, $\text{OH}^-$) and nonradicals ($\text{O}_3$, $\text{H}_2\text{O}_2$, $^{1}\text{O}_2$) that are converted into free radicals. These compounds have different reactivity, hydroxyl radical ($\text{OH}^-$) being the most reactive. Due to the high reactivity of ROS, their interaction with biomolecules may be deleterious to a cell. RPE cells are especially prone to generate an excess of ROS because of their high consumption of oxygen, their exposure to blue light, and their phagocytic function which is accompanied with respiratory burst – an ejection of ROS. An imbalance between production and elimination of ROS may have detrimental consequences to tissues. Normally, cells respond to oxidative stress-induced cellular damages by activating DNA repair systems, by the expression of antioxidant enzymes and molecular chaperones, and by

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(Received June 7, 2013; Revised June 18, 2013; Accepted June 19, 2013; Published online June 24, 2013)
accelerating protein clearance [4]. Failures in these systems lead to a progressive decrease in the clearance of ROS and aggregated proteins in conjunction with chronic inflammation, that are recognized as important factors in the etiology of numerous late-onset degenerative diseases, including AMD [1,4].

3. ROS as a causative factor for AMD

ROS are indispensable for cell survival as they participate in cell signaling, gene expression and cellular differentiation via regulating the cellular redox state or the balance between oxidation/reduction reactions. However, the level of oxidants exceeding the cell’s antioxidant buffering capacity promotes oxidative stress. Generally, oxidative stress is believed to increase with aging [5]. It was reported that aging is predictive of an increased risk of oxidative stress and that it is linked with a progressive and slow decline in antioxidant status in the European free-living healthy elderly [6]. Interestingly, the healthy elderly subjects were not exposed to an acute oxidative stress when compared with middle-aged subjects. The oxidative stress theory of aging, which is particularly enticing in the light of the damaging nature of oxidants in vivo, explains the association between oxidative stress and age-related dysfunction at the level of cell and whole organism. Such phenomena as the association between basal metabolic rate and life expectancy, the accumulation of degenerative disorders in advanced age, and the improvement of life-span with the use of caloric restriction may be easily clarified with this theory.

It is commonly postulated that the cellular antioxidant defense declines and the amount of ROS increases with aging [5, 7]. The rate of aging is established via the amount of cellular structural damage. Also, the retina ROS level elevates and the cellular oxidative damage increases with an age-related tendency. In age-related neurodegenerative diseases, which are associated with the oxidative stress, including Alzheimer’s disease, Parkinson’s disease and AMD, protein and DNA are damaged either directly by ROS or reactive nitrogen species (RNS), or indirectly, by the products of lipid peroxidation [8]. Thus, the accumulation of oxidative damage during lifetime may lead to a dysfunction of RPE cells and increase their susceptibility to exogenous and endogenous insults, eventually culminating in neural cell death and loss of visual function. However, it is difficult to assess whether the augmentation of oxidative damage in AMD is primarily due to elevated level of
ROS or decline in antioxidant defense or a combination of both or alternatively, AMD is a disease related to separate aging-independent pathology.

In vitro studies showed that oxidative stress was generated when cells were exposed to irradiation and that the pre-treatment of cells with ROS scavengers such as vitamins A, C, and E protected against this injury [9]. However, the analysis of the level of these vitamins and the antioxidant status of subjects with/without AMD are not evident since population-based studies of the dietary or plasma levels of antioxidants lack consistency due to the subjectivity of antioxidants nutritional intake estimation or the reflection to recent antioxidants nutritional intake only [10-13]. Although the concentration of another ROS scavenger, α-tocopherol, within the retina is sensitive to its dietary intake, a number of studies manifesting its protective effect seem to be convincing [14-16]. Also high plasma levels of lutein and zeaxanthin, antioxidants, were associated with a reduced risk of neovascular AMD [17, 10]. The excessive amount of iron in the retina may contribute to the pathogenesis of AMD since it may generate ROS through the Fenton reaction. The most convincing study in support of this hypothesis comes from the studies conducted on the post-mortem human retina showing that the concentrations of iron and iron-transporting enzyme, transferrin, were higher in the AMD patients than in sex- and age-matched individuals without visual disturbances [18, 19].

We showed that genetic polymorphism in a number of genes encoding proteins of iron metabolism: NFE2L2 (rs6726395), NOS3 (rs1799983), TF (rs4481157), IRP1 (g.32373708) and IRP2 (g.49520870) may modulate the risk of AMD [20-23]. However, it is still a matter of debate whether iron is the cause of AMD or a byproduct of AMD pathogenesis. A number of above examples are supportive for the hypothesis that AMD is ascribed to the cumulative oxidative stress [10, 13, 15].

4. DNA damage and repair in AMD

4.1. DNA repair decreases with age

There is a growing body of evidence that DNA repair decreases in age-dependent manner. The 30-50% age-related decline in the DNA repair capacity was shown in C. elegans at the whole organism level, suggesting that it may contribute to the age-associated accumulation of DNA damage [24]. With age, the efficacy of base excision repair (BER) and non-homologous end joining (NHEJ) decreased in aging rat neurons [25]. The lowered efficacy of BER was attributed to the deficiency of DNA polymerase β and DNA ligase in aging neurons. The slower rate of the removal of UV-induced DNA lesions and the decreasing levels of proteins that participate in the repair process in aged humans in comparison to younger adults may suggest the decreasing NER function with aging [26-28]. Apart from the observation of age-related diminishing of DNA repair efficacy, the group of disorders characterized by accelerated aging (progeroid syndromes) is related to defects in DNA-processing proteins. This suggests that maintaining the stability of the human genome seems to be of importance in delaying age. In the premature aging patients, e.g. with Werner and Cockayne syndromes, NER-related disorders, DNA damage repair systems were altered, suggesting that an increased accumulation of DNA lesions resulting in premature aging play a causative role in these diseases [29-32]. BER-related human progeroid syndromes are fewer than impaired NER disorders which may be explained by the lethality of embryos with defects in essential BER components or by the multitude of back-up systems, which may reflect the critical role of BER in maintaining genome integrity [33-35].

4.2. DNA repair and AMD

It has been demonstrated that endogenous DNA damage, including DNA strand breaks and alkali-labile sites (ALS) in lymphocytes increases in AMD patients [36]. In addition, the efficacy of DNA repair in AMD patients decreases but in the study of Wozniak et al., there was no difference in DNA repair efficiency between the patients with dry and wet form of AMD. Given that DNA repair may be irregular in AMD, it seems reasonable to consider the efficacy of DNA repair proteins as important factor influencing individual susceptibility to AMD. However, only few studies have been dedicated to this issue [37]. Among DNA repair pathways, BER is the most important for cellular survival in response to oxidative DNA damage since it mainly takes the form of DNA base modifications. The key enzymes participating in BER are DNA glycosylases, which recognize and remove damaged bases. The 8-oxoG glycosylase (human OGG) is responsible for the recognition of 8-oxoG, one of the most stable, toxic, and pre-mutagenic DNA damage of the oxidative origin. The hOGG1 gene encodes 8-oxoguanine DNA glycosylase 1. This enzyme recognizes oxidized bases, removes them and possesses AP (apurinic or apyrimidinic) lyase activity which allows hOGG1 to nick the DNA phosphodiester bond. It was shown that OGG1 was decreased both at mRNA and protein level in aged rodent RPE and choroid [38]. The expression level of hOGG1 decreased almost by half in the macular RPE cells of AMD subjects when compared to that of aged macular RPE cells of healthy controls, suggesting that the protein level of hOGG1 may be reduced in AMD [39]. Reports on the association of the p.S326C polymorphism of the hOGG1 gene with the occurrence of AMD are dubious. One study does not show any relationship between them [40]. In contrast, we demonstrated that the S/C genotype and the C allele significantly increased the risk of AMD in both dry and wet forms and additionally, the S/S genotype and the S allele decreased this risk [41]. Moreover, we showed that the polymorphisms of the SMUG1 (rs3087404) and UNG (rs2337395) genes encoding single-strand-selective monofunctional uracil-DNA glycosylase 1 and UNG uracil-DNA glycosylase, respectively, were associated with the risk of AMD [37]. The c.–32A>G polymorphism (rs3087404) of the SMUG1 gene is located in the non-coding, regulatory region and thus may influence its gene expression through modifying the mRNA stability and degradation. The altered gene expression may in consequence disturb the activity of SMUG1 and thus decrease the DNA protection against oxidative damage. The g.4235T>C (rs2337395) polymorphism of the UNG genes is located in the regulatory region and the
presence of the C/C genotype and C allele increased the risk of dry AMD, but not that of wet form. Interestingly, the C/C genotype decreased the risk of AMD progression from its dry to wet form and the T allele was associated with a deleterious effect. Despite that nucleotide excision repair (NER) is generally considered as a repair system for bulky adducts, it has been demonstrated that NER repairs also non-bulky DNA lesions, such as DNA oxidized bases with moderate efficacy [42, 43]. Thus, NER cannot be taken into account when considering the repair of DNA oxidative-stress-induced damage, but rather should be regarded as having a backup role for BER in the removal of such DNA adducts. The XPD (Xeroderma pigmentosum group D) gene encodes a DNA helicase, which is a component of the core-TFIIH basal transcription factor. It is involved in NER by opening DNA at the site of damage, and in RNA transcription by RNA polymerase II. A relationship between the genetic variants of XPD gene and the AMD occurrence was manifested for two polymorphic sites, p.D312N and p.K751Q, where the 751Q/Q genotype and the 312D-751Q haplotype seemed to have a protective effect against the development of AMD [44]. The ERCC6/CSB (excision repair cross-complementing rodent repair deficiency, complementation group 6) encodes a DNA-binding protein important in the transcription-coupled nucleotide excision repair (TC-NER), which allows removing of RNA polymerase II-blocking lesions from the transcribed strand of active genes. ERCC6 participates in the aging process and DNA repair [45]. Disruption of this gene may be manifested in the ocular degeneration, indicating a possible role of this gene in AMD. The c.-6530C>G polymorphism (rs3793784) is located in the 5' flanking region of this gene and influences different regulation of gene expression in vitro and in vivo. An in silico study demonstrated that its presence might alter the putative transcriptional factor binding patterns around flanking sequences. The C allele corresponds to a possible Sp1 binding element, whereas the G allele corresponds to a possible binding element for Sp1, as well as Oct-1 and GATA-1. The SNP in ERCC6 demonstrated statistical epistasis with the SNP in CFH (rs380390), yielding a combined disease risk OR of 23.05 for individuals homozygous for risk alleles at both the

Fig. 3. A perspective for the regulation of cellular stress response in young versus aged cells. The major signaling controllers have been circled. In young and healthy RPE cells antioxidant defence, quality control of protein folding and regulation of energy metabolism are in balance, while in the aged cells increased oxidative stress, DNA damage and disturbed proteostasis are prominent factors.
CFH and ERCC6 polymorphisms [46]. This biological epistasis may be related to the function of ERCC6, which participates in transcription as a component of RNA pol I transcription complex [45].

Mitochondrial DNA (mtDNA) is more susceptible to oxidative stress-associated damage than nuclear DNA and thus mitochondrial dysfunction may play a pivotal role in AMD pathogenesis [47, 38]. Indeed, the macula-specific increase in mtDNA damage and diminished repair were associated with aging and the severity of AMD [39]. Mitochondrial DNA damage repair in the RPE was relatively slower and less efficient than the repair of nuclear DNA [48]. Taking into account the increased susceptibility of mtDNA to oxidative damage and its weak repair, it may be concluded that lowered mtDNA defenses against oxidative damage in RPE cells are a crucial factor in the pathogenesis of AMD [49]. Moreover, since the mtDNA damage repair is conducted via nucleus-encoded proteins, the changes in nuclear DNA may also affect the maintenance of mtDNA stability.

Nuclear and mitochondrial DNA damages and decreased repair capacity is estimated to disturb proteostasis that coincide with elevated protein damages, misfolding, protein aggregation and impaired clearance in RPE cells [4, 50-52].

5. mTOR and autophagy in the maintenance of RPE cell proteostasis

Autophagy plays multifunctional role in cellular adaptation to stress, including oxidative insults, by maintaining mitochondrial integrity and removing damaged protein [53]. The autophagy process is initiated with the formation of isolation membranes called omegasomes that enlarge via phagophore stage to double membrane autophagosomes that engulf degrading material [54]. Autophagy flux is finalized when autophagosomes fuse with lysosomes and their contents are then degraded by lysosomal enzymes. However, autophagy flux may be impaired in aged postmitotic cells, such as RPE cells [55]. Pharmacological induction of autophagy can enhance the clearance of intracytoplasmic aggregate-prone proteins and ameliorate cellular and tissue pathology [56]. The classical pathway that regulates autophagy acts through the mTOR, a protein kinase that plays a key role as a sensor for energy, nutrients, growth factors, stress and redox changes [57]. mTOR is a ubiquitously expressed, serine/threonine kinase belonging to the phosphoinositide 3-kinase (PI3K)-related kinase family. mTOR inhibition evokes autophagy activation. mTOR consists of a central regulatory catalytic core with two functionally distinct multiprotein complexes, mTOR complex 1 (mTORC1) and complex 2 (mTORC2). Rheb, Raptor, PRAS40-P, mLST8, and Deptor are regulatory units for mTORC1, while Sin1, Rictor, mLST8 and Protor are the corresponding units for mTORC2 [58]. Previous observations show that the rapamycin-induced mTORC1 inhibition and the activation of autophagy can slow down the aging process and preserve retinal cell function [59-62]. At present, there is convincing evidence that the modulation of mTOR may be a potential target for the development of new therapeutic strategies for neurodegenerative diseases including AMD [1, 56].

In addition to mTOR, the insulin/insulin-like growth factor 1 (IGF-1) and AMP-activated protein kinase (AMPK) pathways, as well as sirtuins are included in the signaling mechanisms that control the stress response and DNA repair systems in cells [52] (Fig. 3). Despite of its important role in enhancing growth during development, insulin/IGF-1 signaling can potentiate aging by inhibiting autophagy through the activation of mTOR via PI3K and Akt [63]. Moreover, the insulin pathway can inhibit Forkhead box O (FoxO) transcription factors, which could otherwise promote autophagy [64-67]. FoxO proteins are evolutionarily conserved regulators downstream from insulin/IGF-1 receptors that control central cellular functions, such as cell cycle, cellular metabolism and cell death [68]. They can be mutually activated by SIRT1 and AMPK [66,69-71]. SIRT1 is a nicotinamide adenine dinucleotide (NAD\(^+\))-dependent deacetylase, which function has been associated with increased longevity [72]. SIRT1 also supports autophagy through AMPK, which further activates SIRT1 by a positive feedback mechanism [73-74]. Peroxisome proliferator-activated receptor-\(\gamma\) coactivator (PGC-1\(\alpha\)) activated by AMPK, in turn, contributes to mitochondrial biogenesis and thereby inhibits the oxidative stress. This defense response may simultaneously be supported with a crosstalk of endoplasmic reticulum (ER) and mTOR signaling that reduce ER stress and prevent AMD development [75-77].

6. Discussion and perspectives

The correlative relationship between oxidative stress and AMD is strong and the causative role of oxidative stress in the onset and progression of AMD is convincing. The excess of ROS pose a threat to both DNA and proteostasis. Future work should be aimed at the research on the significance of the genetic variation in the proteins responsible for recognizing and removing oxidative damages. DNA repair-oriented therapy could support currently applied antioxidant therapeutic strategies and could become a part of a multifaceted and personalized approach in the treatment of AMD. Since AMD is also a protein aggregation disease, it should be appreciated that autophagy may represent an important therapeutic target in AMD. In particular, the autophagy-regulating kinases AMPK and mTOR can be potential therapeutic targets for preventing RPE cell degeneration and AMD progression.

Acknowledgements

This work was supported by the EVO (grants of Kuopio University Hospital, the Finnish Cultural Foundation and its North Savo Fund (KK), the Finnish Eye Foundation (KK), the Finnish Funding Agency for Technology and Innovation (KK), Health Research Council of the Academy of Finland (KK, AK), and the Päivikki and Sakari Sohlberg Foundation (AK).
Conflict of interest: None declared.

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ISSN 2168-8761 print/ISSN 2168-877X online ~ 111 ~ http://www.researchpub.org/journal/jbpr/jbpr.html


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