



Routes of Oxidative Stress in Age-Related Macular Degeneration

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Abstract

Oxidative stress has a critical role in the pathogenesis of age-related macular degeneration (AMD), a multifactorial disease that includes age, gene variants of complement regulatory proteins and smoking as the main risk factors.

The pathogenic mechanism of AMD is poorly understood. The ROS and nitrogen species produced during stress may damage crucial biomolecules of the retinal pigment epithelium (RPE) cells. There is a strong relation between stress oxidative and inflammatory response in the pathology of the AMD. Actually, there are many routes of oxidative stress related with AMD but the most representative are: autophagy/heterophagy, apoptosis, and iron, DNA and antioxidant enzymes. Autophagy is a cellular housekeeping process that removes damaged organelles and protein aggregates, whereas heterophagy, in the case of the RPE, is the phagocytosis of exogenous photoreceptor outer segments. Numerous studies have demonstrated that both autophagy and heterophagy are highly active in the RPE. Furthermore, the overexpression of CHOP can lead to cell cycle arrest and apoptosis, and activation of caspase cascades also occurs in ER stress. The α B crystallin is much studied because it has been shown to have antiapoptotic properties in RPE cells. Other possible cause is the excess iron in patients diagnosed AMD because induces tissue damage. These effects have been confirmed in rodent research with the downregulation of DNA repair enzymes involved in repair of oxidative damage occurred in aged RPE and choroid. Finally, the genetic defects (antioxidant enzymes), dietary or uptake deficiencies in antioxidants, or exposure to noxious agents (cigarette smoking) could enhance oxidative RPE damage during life and predispose to AMD. There are many routes related with oxidative stress and inflammatory response related with AMD patients. Antioxidant supplementation helped attenuate the development of choroidal new vessels but it is necessary to invest all mechanism of this ocular pathology to develop new therapies.

Introduction

The age-related macular degeneration (AMD) is the leading cause of blindness in elderly populations. Epidemiological studies over the last two decades have provided the risk factors associated with AMD, including: age, sex, diet, nutrition status, smoking, and genetic markers [1].

The AMD can be divided into two main forms: dry (atrophic) and wet (exudative) type and further subdivided into early and late stage disease. The early stage of dry AMD is asymptomatic, although

pigment mottling, accumulation of intracellular lysosomal lipofuscin, and extracellular drusen deposits can be detected [2]. In wet AMD, aberrant blood vessels sprout from the choroidal capillaries (choroidal new vessels, CNV) and penetrate through the Bruch's membrane leading to subretinal membranes, hemorrhage, retinal edema and damage to retinal cells. If left untreated, late stage fibrosis and permanent visual loss may occur.

The mitochondria may be especially important in this process because the reactive oxygen species (ROS) produced in their electron transport chain can damage cellular components. In several studies, the increase in mitochondrial DNA (mtDNA) damage and mutations (for example, production of oxidized derivatives of the DNA bases, 8-oxoguanine), and the decrease in the efficacy of DNA repair have been correlated with the occurrence and the stage of AMD. Also, lymphocytes from AMD patients displayed a higher amount of total endogenous basal and oxidative DNA damage, exhibited a higher sensitivity to hydrogen peroxide and UV radiation, and repaired the lesions [3]. Furthermore, there is increasing evidence that constant oxidative stress impairs autophagy and heterophagy, as well as increases protein aggregation and causes inflammasome activation leading to the pathological phenotype of AMD. Other mechanism is the iron accumulation in AMD, that it is a toxic in the visual phototransduction cascade and catalyzes the conversion of hydrogen peroxide to hydroxyl radical, which is the most damaging of the ROS. This has therapeutic potential for diminishing iron-induced oxidative damage to prevent or treat AMD.

Dietary factors play an important role in the control of stress oxidative. In a large clinical trial, dietary supplements of antioxidants including carotenoids, vitamin C, vitamin E and zinc, experienced reduced progression to advanced AMD [4]. The effect of lutein was a significant reduction in malondialdehyde (MDA) level and increase in the gene expression of antioxidant enzyme activities (SODs and catalase).

Finally, it is very important to know the pathogenic mechanism of AMD so the population needs new therapies to prevent and treat exudative and atrophic maculopathy. The use of antioxidant vitamins has been shown to delay disease progression at an intermediate stage, and rapid innovation in the use of antiangiogenic therapies has resulted in new clinical methods to treat the exudative phase of AMD. However, developing new treatment and prevention strategies targeting earlier stages of the disease requires a better understanding of the underlying disease mechanisms.

Stress Oxidative in AMD

The pathogenic mechanism of AMD is poorly understood. The production of reactive oxygen species (ROS) is stimulated by irradiation, aging, inflammation, increased partial pressure of oxygen, air pollutants, cigarette smoke, and reperfusion injury. The oxygen-derived metabolites cause oxidative damage to cytoplasmic and nuclear elements of cells and cause changes in the abnormal extracellular matrix [5].

We will collect the different routes studied oxidative stress in AMD, and also add recent studies provide important data in this clinical area.

Autophagy and heterophagy

Evidence of inflammatory cell involvement in the later stages of AMD includes the presence of multinucleated giant cells and leukocytes in the choroid of AMD. Therefore, there is a strong relation between stress oxidative and inflammatory response in the pathology of the AMD.

The RPE constitutes the outermost layer of the retina, and has many important functions in the homeostasis of the eye to maintain normal vision. The photoreceptor outer segments are enriched in polyunsaturated fatty acids, which can undergo lipid peroxidation. It is important to recognize aging changes in the RPE-Bruch membrane-choriocapillaris complex that occur in aged eyes. In vitro evidence [5] indicates that RPE lipofuscin is a photo inducible generator of ROS that can compromise lysosomal integrity, induce lipid peroxidation, reduce phagocytic capacity and cause RPE cell death. These oxidative processes are thought to contribute to the clinical manifestation of pigment dispersion, accumulation of intracellular lysosomal lipofuscin and extracellular drusen deposits [6].

When there is damage in the autophagy and/or heterophagy produce the accumulation of auto-oxidative lipofuscin in the lysosomes of RPE cells, as well as drusen formation in the extracellular space between the RPE and the Bruch's membrane, this situation is increased with the aging. Furthermore, the oxidized low-density lipoproteins and lipid peroxidation reduce the degradation of phagocytosed photoreceptor outer segments material, and increase cellular stress in the RPE cell [7]. It has been speculated that lipofuscin itself could be cytotoxic because of its ability to provide a redox-active surface by incorporating oxidatively labile iron [8]. This could result in the formation of oxygen radicals and cause oxidative modification of proteins, lipids and nucleic acids. As a result, the high metabolic activity impaired mitochondrial function is assumed to lead to the accelerated degeneration of RPE cells and secondarily to photoreceptor cell death [9].

The β A3/A1-crystallin is a novel lysosomal component that could regulate both phagocytosis and autophagy to reduce the oxidative stress. The α B crystallin has been shown to have antiapoptotic properties in RPE cells [10]. In AMD, α B crystallin is expressed in RPE in association with subretinal drusen deposits and it is more prominently expressed in the late stage of the disease. Deficiency of α B crystallin sensitizes RPE cells and other cells to external stress and the α B crystallin overexpression protects RPE from stress induced apoptosis [11]. Sinha *et al.* [12] demonstrated that β A3/A1-crystallin localizes to lysosomes in the RPE and that it is a binding partner of V-ATPase, the proton pump that acidifies the lysosomal lumen. This suggests that β A3/A1-crystallin may also be a potential target for therapeutic intervention in AMD.

Iannaccone *et al.* [13] identified targets may be mechanistically linked to AMD pathogenesis because the identified proteins are implicated in autophagy, immunomodulation, and protection from oxidative stress and apoptosis. In particular, a role in autophagy activation is shared by all five auto antigens, raising the possibility that the detected autoantibodies may play a role in AMD via autophagy compromise and downstream activation of the inflammasome.

In conclusion, a failure of the RPE cells to use autophagy can result in accumulation of aggregation-prone proteins, cellular degeneration and finally cell death. There is increasing evidence that constant oxidative stress impairs autophagy and heterophagy, as well as increases protein aggregation and causes inflammasome activation leading to the pathological phenotype of AMD [14].

Apoptosis

The available evidence suggests that transcriptional induction of CHOP is critical in ER stress induced apoptosis. Overexpression of CHOP can lead to cell cycle arrest and apoptosis, and activation of caspase cascades also occurs in ER stress.

A study to investigate the relation the apoptosis and cigarette smoke. The present study aimed to investigate the role of stress and the unfolded protein response in cigarette smoke related RPE apoptosis [15]. The inhibition of CHOP activation by pharmacological chaperones or genetic approaches attenuated hydroquinone-induced RPE cell apoptosis. In contrast to enhanced CHOP activation, protein level of active XBP1, a major regulator of the adaptive unfolded protein response, was reduced in hydroquinone-treated cells. Conversely, overexpressing XBP1 protected RPE cells and attenuated oxidative stress-induced RPE apoptosis. The expression of α B crystallin has also been linked to AMD, where its increased expression has been suggested to be a biomarker for the disease. In the actually study evaluated the role of α B crystallin in ER stress-mediated apoptosis in RPE, and demonstrate that α B crystallin provides critical protection of mitochondrial function in this process [15].

Other recent study [16] observed the activity of factor H which binds apoptotic cells to limit the inflammatory potential of complement. The factor H is actively internalized by apoptotic cells to enhance cathepsin L-mediated cleavage of endogenously expressed C3, which results in increased surface opsonization with iC3b. In addition, internalized FH forms complexes with nucleosomes, facilitates their phagocytosis by monocytes and induces an anti-inflammatory biased cytokine profile. To sum up, this factor diminishes the immunogenic and inflammatory potential of autoantigens.

This section is very important to discover new therapies, for example: histone deacetylases (HDACs). HDACs are a major component of this system and serve as a unique control of the chromatin remodeling process. With a multitude of targeted HDAC inhibitors now available, their use in both basic science and clinical studies has widened substantially. Berner *et al.* [17] suggested that HDAC inhibition may suppress neovascularization and may be a possible treatment for retinitis pigmentosa and AMD.

The α A- and α B crystallins (we had named in the section of autophagy) are principal members of the small heat shock protein family and elicit both a cell protective function and a chaperone function. α -Crystallins have been found to be prominent proteins in normal and pathological retina emphasizing the importance for in-depth understanding of their function and significance. The availability of chaperone-containing minipeptides of α B crystallin could prove to be a valuable new tool for therapeutic treatment of retinal disorders. Finally, the use of α -crystallin derived peptides is a promising therapeutic strategy to combat retinal diseases such as AMD [18].

Iron

Excess iron induces tissue damage and is implicated in age-related macular degeneration (AMD). Iron toxicity is widely attributed to hydroxyl radical formation through Fenton's reaction. Iron-induced degeneration of the RPE is suppressed in mice lacking inflammasome complement component 3 (C3), a central protein in the complement cascade. Li *et al.* [19] showed herein that mutation of this domain reduced iron-induced C3 promoter activity. The molecular events in the iron-C3 pathway represent therapeutic targets for AMD or other diseases exacerbated by iron-induced local complement dysregulation.

Studies detected increased iron in AMD affected maculas, specifically in the RPE and Bruch's membrane of early AMD, geographic atrophy and exudative AMD patients. In the *study Post-mortem*, AMD eyes have increased levels of iron in retina compared to age-matched healthy donors. The iron chelation protects photoreceptors and RPE in a variety of mouse models. This has therapeutic potential for diminishing iron-induced oxidative damage to prevent or treat AMD [20]. Other study *post-mortem*, RPE from patients with early AMD exhibited decreased Bmp6 (is important for regulation of iron homeostasis) levels and contributes to iron build-up in AMD [21]. Other recent study investigated whether iron can also be stored in specialized metal-binding melanosomes of the RPE and choroid and in age pigments of the RPE (lipofuscin and melanolipofuscin). As accumulation of debris in Bruch's membrane is an additional hallmark of AMD, the elemental composition of Bruch's membrane was also investigated. The elastic layer of Bruch's membrane of all AMD donors also contained significantly higher iron mole fractions compared to controls, predominantly in areas that were also rich in calcium and phosphorus, suggesting calcification. In conclusion, iron accumulation in melanosomal storages and within calcified Bruch's membrane is more pronounced in donors suffering from AMD compared to age-matched controls [22].

DNA

The ROS and nitrogen species produced during stress may damage crucial biomolecules of the RPE cells. These effects have been confirmed in rodent research with the downregulation of DNA repair enzymes involved in repair of oxidative damage occurred in aged RPE and choroid. Over 600 genes showed significant differences in expression between the two strains. These genes are involved in disease-associated pathways such as immune response, inflammation, apoptosis, Ca²⁺ homeostasis and oxidative stress [23]. Also, the oxidative stress may be associated with many kinds of DNA damage, including single- and double-strand breaks and oxidative modifications of the DNA bases. For example, 8-Oxo-7,8-dihydroguanine (8-oxoG) is one hallmark of oxidative DNA damage and a major promutagenic component in oxidative stress [24].

The expression of the *RAD51* gene, the product of which plays a crucial role in the repair of DNA double-strand breaks by homologous recombination, was down-regulated in response to an oxidative stimulus in AMD. Also, lymphocytes from AMD patients exhibited a higher sensitivity to hydrogen peroxide and UV radiation, and repaired the lesions induced by these agents less efficiently than the corresponding cells from the control individuals. We postulate that the impaired efficacy of DNA repair may be combined with the enhanced sensitivity of RPE cells to blue and UV light, contributing to the pathogenesis of AMD [25]. Other gene is the signal transducer and activator of transcription-3 protein (STAT3) for the regulation of cell differentiation, proliferation, and angiogenesis. Previous studies have verified that STAT3 is a direct transcriptional activator of the vascular endothelial growth factor (VEGF) gene. Recent findings have suggested that diabetes increases the level of STAT3 activation and thereby contributes to the pathophysiology of vascular injury. These results suggest that hyperglycaemia promotes the development of CNV by inducing oxidative stress, which in turn activates STAT3 signalling in RPE cells. Antioxidant supplementation helped attenuate the development of CNV [26].

Also, the tumour inhibitor p53 gene has the ability of triggering proliferation arrest and cellular death by apoptosis subsequent to several factors, among them oxidative stress. The p53 protein is a major regulator of gene expression. Using genetically manipulated mice carrying an extra copy of gene p53 (transgenic mice super p53) versus control mice, we have investigated the generation of ROS and antioxidant activity in the optic nerve of mice in relation to p53 availability. A significant increase in free radical formation, antioxidant activity and nitric oxide synthesis was found in the optic nerves from transgenic super p53 mice compared to respective controls. The presence of an extra copy of the p53 gene correlated

with redox status in the mouse optic nerve. This transgenic mouse could be useful as an experimental model to study cell resistance to neurodegenerative processes in relation to oxidative stress and to apoptosis induction, such as AMD [27].

Other genetic study is the novel gene *HERPUD1* involved in the polypoidal choroidal vasculopathy. *HERPUD1* has been reported to increase the level of amyloid β , which is a component of drusen deposits underlying RPE layer. To verify the genetic functional associations of *HERPUD1* with polypoidal choroidal vasculopathy, exome sequencing of *HERPUD1* was performed in unrelated Chinese individuals. Jin *et al.* [28] showed that *HERPUD1* is significant associated with neovascular AMD, and amyloid β could upregulate angiogenic factors, chemokines and matrix metalloproteinases both in RPE cells. The imbalance of the cytokines may be one of the mechanisms for the formation and development of neovascular AMD.

Actually, there are many lines of investigation to look for genes involved in the pathology of AMD. We had named the most representative.

Antioxidants: enzymes and supplements

To combat the deleterious effects of ROS, cells have evolved an intrinsic antioxidant defense network that consists of a variety of scavengers, such as superoxide dismutase (SODs), glutathione peroxidase (GPx-s), catalase and glutathione S-transferase. The level of the enzymes is decrease in AMD, for example: catalase and glutathione reductase, SODs, metallothionein (an acute-phase reactant protein that scavenges hydroxyl radicals), heme oxygenase-1 and heme oxygenase-2), etc. The genetic defects (e.g. in antioxidant enzymes), dietary or uptake deficiencies in antioxidants, or exposure to noxious agents (e.g. cigarette smoking) could enhance oxidative RPE damage during life and predispose to AMD [3].

Other enzyme is the paraoxonase (PON1) is essential for protection of LDL against atherogenic oxidative damage. The homocysteine (Hcy) can be converted into homocysteine thiolactone in all cell types; this leads to potentially harmful protein damage by homocysteinylation that may be involved in the pathology of vascular diseases. PON1 shows homocysteine thiolactonase activity, which might contribute to the detoxification of this metabolite of Hcy. The patients with exudative-type AMD had significantly higher Hcy concentrations compared to controls. The higher Hcy levels were correlated with higher serum malondialdehyde, and lower PON 1 activity; according to results, it can be stated that Hcy may negatively regulate PON 1 gene expression in active AMD, which could contribute to lower serum PON1 activity due to enhanced oxidative stress [29].

Furthermore, the antioxidant enzyme methionine sulfoxide reductase A (*MsrA*) is highly expressed in the RPE. Study tested the effects of acute silencing or overexpression of *MsrA* on the phagocytosis of photoreceptor outer segment fragments, a demanding, and daily function of the RPE that is essential for vision. RPE cells manipulated to express higher or lower levels of *MsrA* than control cells showed no signs of cell death but increased or decreased, respectively, POS binding as well as engulfment. Furthermore, ATP content directly correlated with *MsrA* protein levels in RPE cells that used mitochondrial oxidative phosphorylation for ATP synthesis but not in RPE cells that relied on glycolysis alone. The contribution of *MsrA* to energy metabolism is independent of its function in protection from elevated oxidative stress but contributes to routine but vital photoreceptor support by RPE cells [30].

The astaxanthin (AST) has been shown to have a high level of antioxidant activity 10 times higher than that of other carotenoids, such as lutein, canthaxanthin, and β -carotene and 100 times higher than α -tocopherol. The treatment with AST reduces H₂O₂-induced cell death and apoptosis in cells, and that the mechanism by which AST induced cytoprotection could include the Nrf2-antioxidant-response element. These data indicate that AST might provide a valuable therapeutic strategy for early AMD [31]. Other sustain is

the canolol, which exhibits potent antioxidant activity. In the study, the investigators found that there was an increase in the expression of catalase and glutathione S-transferase-pi upon canolol treatment, which was positively correlated with the protective effects of canolol [32]. Finally, the melatonin has potential to prevent telomere shortening in RPE, while not precluding other mechanisms, namely antioxidative properties and/or restoration of inner blood-retina barrier integrity, reduced VEGF, hypoxia and nitric oxide levels [33].

Other antioxidant activities studied are: vitamins A, C and E, beta-carotene, omega-3 fatty acids (alpha-linolenic acid, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)), and some minerals, such as zinc, copper, selenium and manganese.

In 2001, AREDS report no. 8, a large multicentric, randomized clinical trial, revealed that the risk of progression to advanced AMD was reduced by 28% in patients with intermediate AMD treated with high doses of antioxidant supplements (vitamins C and E, zinc and β -carotene) [34]. In 2004, AREDS report no. 13 showed that mortality was lower in patients taking zinc alone or with antioxidants when compared to those not taking this mineral [35]. The Rotterdam Study showed that an above-average intake of the 4 AREDS trial nutrients protected against AMD development or early AMD, as indicated by large drusen, and was associated with a 35% reduction in the risk of AMD [36]. In 2015, AREDS report no. 37 observed that the increased dietary riboflavin and B12 were associated inversely with nuclear and cortical lens opacities. For participants taking multivitamins during the study, the highest intake of dietary folate was associated with an increased risk of mild posterior subcapsular lens opacity development. No statistically significant associations were found between lutein plus zeaxanthin intake and presence at baseline or development of nuclear or cortical lens opacity outcomes [37].

Zinc is an important trace metal in retina and its deficiency leads to AMD. Recent studies on zinc sulphide nanoparticles (ZnS-NPs) are gaining attention in the field of physical and biological research. In this study [38] observed that ZnS-NPs stabilize reactive oxygen species elevation, when subjected to hydrogen peroxide- and thapsigargin-mediated oxidant injury and helps in maintaining normal homeostasis through regulating ER stress response proteins which is the lead cause for apoptosis-mediated pathogenesis of AMD.

The xanthophyll carotenoids lutein, zeaxanthin and meso-zeaxanthin are found at the macula, the central part of the retina, where they are referred to as macular pigment. Silvan *et al.* [39] designed to measure the egg yolk carotenoid response to hen supplementation with lutein, zeaxanthin and meso-zeaxanthin. The results inform attempts to develop a novel food designed to increase macular pigment, and its constituent carotenoids in serum, and confer associated health and visual benefits. Other study with cyanidin-3-O-glucoside, lutein and zeaxanthin demonstrated that this supplement is effective in preventing UVB-induced damage in RPE cells and may be suitable as chemoprotective factors for the prevention of ocular damage [40].

Conclusion

The pathogenic mechanism of AMD is poorly understood. An imbalance between the production and neutralization of ROS by antioxidant defence is associated with oxidative stress, which plays an important role in the pathogenesis of many age-related and degenerative diseases.

The use of natural dietary antioxidants might reduce ocular oxidative damage but it is necessary to invest new routes related with the different causes of oxidative stress to develop new drugs in AMD patients.

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