Molecular and cellular mechanisms of retinal degeneration in macular dystrophy

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Abstract

Among macular dystrophy, the Age-related macular degeneration (AMD) is the leading cause of the irreversible blindness in the industrially developed countries, affecting approximately 25% of people over the age of 65 years. AMD is characterized by the progressive loss of central vision and is the result of degenerative changes in the central region of the retina, the macula. Major risk factors such as age, smoking, arterial hypertension, hyperopia, coronary artery diseases, lens opacity or previous cataract.

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surgery have been characterized. As with other diseases of late onset, the etiology of AMD is almost certain to be polygenic and multifactorial. Epidemiologic and genetic studies have suggested that both environmental and inherited factors increase the risk of AMD. But to date, no gene mutations have been directly involved in AMD.

There are two forms of AMD, the dry nonexsudative or atrophic form and the exsudative or wet form. It has been hypothesized that the progressive loss of vision associated with the nonexsudative form of AMD is attributable to the accumulation of extracellular deposits, known as drusen, between the RPE and the Bruch’s membrane. As drusen increase in size and number, they compromise the function and survival of the RPE, which is absolutely required for photoreceptor maintenance. It has been hypothesized that retinal degeneration is therefore secondary to age-related alterations in RPE support function, in AMD. Because RPE accumulates lipofuscin, a marker of senescence, impairing RPE function, such as lysosomal activity, during AMD, it has been postulated that lipofuscin may contribute to AMD. In this review are described the recent theories based on epidemiological studies of diet, or biochemical and molecular studies on the role of drusen, light-induced oxidative damage (including blue light’s damaging potential and the role of A2E), inflammatory and immune-mediated events that have been also postulated to explain RPE cell death in AMD. These hypotheses are not exclusive. Such novel insights into the possible causes of AMD might give rise to preventative and/or reconstructive treatments in the future. For example, therapeutic or nutritional intervention to enhance the antioxidant capacity of RPE and photoreceptors may provide an effective way to prevent, delay the onset or treat AMD. Manipulation of chemotactic cytokines and/or their cognate receptors, such as MCP-1-Ccr2 chemoattractant protein pathway, to inhibit recruitment of macrophage late in the course of AMD to reduce the degradation of Bruch’s membrane and avoid angiogenesis, might be also beneficial. In addition, characterization of the cellular and molecular events involved in the passage of normal aged retina to the pathological degenerative retina will help to identify key factors and molecules on which therapeutic intervention will help to prevent or decrease the time course of AMD. Description and understanding the molecular events involved in retinal degeneration, such as apoptosis and/or necrosis of RPE cells and photoreceptors by different injuries, which were postulated to be involved in retinal degeneration in AMD, are essential to develop future antidegenerating strategies. The recently described role of neurotrophic growth factors and cytokines, such as cone survival factors, and natural derivative of fatty acids such as NPD1, on the protection of photoreceptors or RPE cells will also help to develop potential future ways to combat neurodegenerative processes. Identification of their precise mode of action, including the intracellular
signaling targeting the antiapoptotic pathways will certainly contribute to the development of new therapeutic strategies against AMD.

I. The structure of the mammalian retina

The retina, an extension of the brain, forms the inner lining of the posterior eyeball. It is composed of a neural and a pigmented retina, the retinal pigment epithelium. Retina is the only part of the central nervous system that is directly exposed to light. The neural retina converts light energy to electrochemical signals through the phototransduction cascade, for transmission to the brain. The vertebrate neural retina is a highly organized tissue which consists of five types of neurons and one type of glial cell. The majority of synapses are confined to two synaptic areas, the outer and the inner plexiform layers. The cell bodies of the neural retina are organized into three nuclear layers: the ganglion cell layer which contains the ganglion cells and the displaced amacrine cells, the inner nuclear layer which contains the bipolar, horizontal and amacrine cells, and the outer nuclear layer which contains the two types of photoreceptors, the rods and the cones. There are approximately 150 million photoreceptors in the adult human retina. Rods are responsible for vision in dim monochromatic light, therefore allowing night vision, while cones are adapted to photic conditions and the perception of the color.

Retina consists of two distinct regions, the central retina or macula and the peripheral retina. The macula is an oval area at the posterior pole measuring about 5 mm in diameter. Important clinical landmarks within the macula are the fovea (1.5 mm in diameter) which is a depression in the inner retinal surface at the center of the macula, and the foveola (0.35 mm in diameter) which forms the central floor of the fovea. The foveola is devoid of ganglion cells and its entire thickness consists only of cones. Rods are the major component of the peripheral retina, with a maximal density around the perimacular region. The macula contains two sub-regions with distinctly photoreceptor content: the cone-dominated fovea and a rod-dominated parafovea [1]. In young adults, rods outnumber cones in the macula by 9:1, and in the entire retina, rods outnumber cones by 20:1 [2]. The concentration of cones is maximal at the fovea centralis, an area associated with the ability to visualize fine details. Therefore, macula is responsible for our fine visual acuity and color vision.

The photoreceptor outer segment is an appendage filled with stacked membranes containing proteins of the phototransduction signaling, called Disks. Disks contain the photoactive pigment opsin, and the chromophore, 11-cis retinal. Photoreceptors shed as many as 100 discs per day. They are continually replaced by the addition of new disks at the base of the stack. The distal tips of the photoreceptor outer segments are daily phagocyted and degraded by the retinal pigment epithelium (RPE). Each RPE cell ingests as
many as 4000 outer segment disks per day. The inactivated chromophore is converted in the presence of vitamin A to 11-cis retinal and transported back to the photoreceptors.

In adult, RPE consists of a single sheet of post-mitotic cells that lines the inside of the eye between the photoreceptors and their supporting vascular network of large capillaries, the choriocapillaris. Choriocapillaris supply the RPE and underlying photoreceptors with oxygen and nutrients. The choriocapillaris has the highest blood flow in the body. The RPE forms the equivalent of the blood-brain barrier between the photoreceptors and the choriocapillaris. It performs a number of functions that are essential to maintaining photoreceptor cell functions and survival. RPE sustains photoreceptor health by maintaining proper ionic balance and hydration, delivery and filtering nutrients and metabolites to the photoreceptors. In particular, RPE is essential in the conversion of all-trans retinol form of vitamin A in the blood to the 11-cis retinaldehyde required for visual pigment synthesis in photoreceptors. RPE is required for development and maintenance of the neural retina [3]. Impairment of the RPE-mediated functions results in loss of photoreceptors [4]. Between the RPE and the choriocapillaris is Bruch’s membrane that consists of five layers: the RPE basal lamina, the inner collagenous layer, elastic layer, outer collagenous layer and choriocapillary endothelium basal lamina. Photoreceptor, RPE and choriocapillaris form a functional unit.

II. Aging of the retina
1. Structural, biochemical and functional changes of photoreceptors

Changes in visual signal of the retina during normal aging have been observed. Several findings demonstrate the functional vulnerability of rod during aging: 1- scotopic impairment was greater than photopic impairment, 2- scotopic sensitivity declines throughout adulthood faster than photopic sensitivity and 3- the delayed dark adaptation in the elderly is the result of the altered kinetics of rod function [5,6]. In addition functional changes have been observed in different cell types in normal retina during aging: the waveform of the electroretinogram changes, due to slowed temporal adaptation [7] and the inner retinal function and the rod-cone interaction decreased with age [8]. Structural alterations have been also observed in the human retina during aging. Among them, is the photoreceptor cell loss, with the rods being more affected than are the cones [9,10]. About half of the rods are lost between the second and the fourth decades, with a rate of annual disappearance of 970 cells per mm² in the peripheral retina [10]. In the central retina, the density of rods decreases by 30% between the ages of 34 and 90 years, while the number of
cones remains stable in the macula [9]. Because it has been shown that cones survival may depends of the presence of rods, even if they are not functional, due to the secretion of cone survival factors [11-13], a similar process may be involved during normal aging and in AMD, in which more rods are loss than cones [14].

2. Structural, biochemical and functional changes of RPE and Bruch’s membrane

RPE also undergoes a variety of structural and biochemical changes with age, which are generally considered to be detrimental to optical cell function. Age-related structural RPE cell changes include loss of cell shape, hyperplasia with regions of multilayered cells, hyperpigmentation, atrophy and increase in cell diameter as cells spread to fill the spaces left by dead RPE cells [15]. RPE cell density decreases by up to 0.3% per year with increasing age [16]. In the aging eye, apoptosis of RPE cells is four times higher in the macular center than in the rest of retina [17]. It has been estimated that nearly 20% of the macular RPE would be lost per decade in older human eyes [17]. In addition, RPE cells express senescence-related β-galactosidase (SA β-gal) and lose telomerase during aging [18,19] SA β-gal staining in RPE is adjacent to drusen in eyes of old, but not of young primates. Interestingly, cultured human RPE cells that have been transfected with human telomerase gene (hTERT) are resistant to senescence and do not express SA β-gal activity [20]. But no correlation between the proportion of senescent cells in RPE, disease and telomere length in AMD has been demonstrated to date. The ability of the RPE to mediate photoreceptor outer segment turnover seems to be reduced during aging [21]. Vitamin A metabolism by the RPE is also altered during aging [22].

Accumulation of intracellular deposits within cells is also a hallmark of retina aging. Significant pigmentary changes in the RPE have been observed. These changes include a linear increase in lipofuscin granules, a decrease in melanosome numbers and an increase in pigment complexes (melanolipofuscin and melanolysosomes).

2a. Lipofuscin

Lipofuscin is a complex of autofluorescent, lipid and protein aggregates. It is generated within the lysosomal system of a variety of post-mitotic cells and is referred to as a senescent marker. Retinal cells from older individuals have higher accumulation of age-related pigment lipofuscin, than most older tissues in the body [23]. The common substrate for lipofuscin formation is autophagy of spent intracellular organelles such as golgi bodies, endoplasmic reticulum and mitochondria. RPE lipofuscin is derived primarily from components of the
phagocytosed photoreceptor outer segments. It contains retinoid metabolites derived from the ingestion of outer segment tips [24]. In the RPE lipofuscin granules are typically concentrated around the nucleus in the basal half of the cell. In the first decade of life, lipofuscin occupies just 1% of the RPE intracellular space. During aging, lipofuscin occupies a larger fraction of the RPE cell volume (up to 19% of cytoplasmic volume by 81-90 years of age) [25]. Age-related accumulation of lipofuscin in the RPE is accompanied by changes in its morphological features as well as in the morphology of its extracellular matrix [25-27]. Although a cause-and-effect relationship between RPE lipofuscin accumulation and altered function cannot be demonstrated, there is a direct correlation between RPE lipofuscin accumulation, impaired RPE cell function and consequent photoreceptor damage and loss. All these three factors are correlated with aging. Because, lipofuscin is exposed to high tensions of oxygen and to visible light, it is a generator of reactive oxygen species (ROS). Despite considerable research effort, the chemical identity of the main photosensitizers present in lipofuscin remains unknown. One of the components of lipofuscin that is considered as a potential photosensitizer is the orange fluorescent pyridinium bisretinoid, A2E (N-retinylidene-N-retinylethanolamine). Following exposure to light, lipofuscin generates superoxide ions, singlet oxygen, hydrogen peroxide and lipid peroxides [28]. Therefore, lipofuscin may participate to the damage of proteins, lipid membranes and nuclear and mitochondrial DNA within retina cells during aging.

Three possible mechanisms may exist whereby lipofuscin may disrupt RPE cellular function and may participate to RPE cell degeneration during aging and in AMD. Distortion of cellular architecture and reduction in functional cytoplasmic space, resulting from the accumulation of lipofuscin granules, may compromise the metabolism of the cell. Alternatively, lipofuscin and particularly, A2E, may induce oxidative damage because it acts as a photosensitizer for generation of ROS. Finally, RPE lysosomal degradative function may be inhibited by A2E, limiting the cell’s capacity to digest intra- and extra-cellular materials. (See detailed mechanisms in section 3b).

2b. Melanosomes

The pigmentary changes in RPE also include a decrease in melanosome numbers and an increase in pigment complexes (melanolipofuscin and melanolysosomes). Production of melanin occurs throughout life in RPE. Age-related changes in melanosomes include disorientation within the RPE, a decrease in number after the age of 40 years [25,29], loss of melanin [30] and an increase in melanosome complexes with lysosomes and/or lipofuscin [25]. Photophysical characteristics of melanosomes also change with age, with an increased absorption at the shorter wavelengths and a decrease in the blue in
fluorescent spectra [31]. Moreover, aged melanosome are highly photoreactive, resulting in oxidative damage of RPE, whereas young melanosomes confer photoprotection [32,33].

2c. The Bruch’s membrane and choroid

Alteration of the Bruch’s membrane has been also observed during aging of the retina. Age-related hydrodynamic changes of Bruch’s membrane are observed. Bruch’s membrane thickness increases with age (over 135% over 10 decades), therefore increasing the diffusional path length [34]. The permeability of Bruch’s membrane to serum proteins decreases by 90% over 90 years [35]. A direct relationship between Bruch’s membrane thickness and lipofuscin accumulation within RPE has been demonstrated in old eyes [36]. Characteristic debris accumulates within Bruch’s membrane throughout adulthood [37], accompanied by reduced collagen solubility (decline by 50% over 90) [38], and deposition of neutral lipids [39]. The progressive increase in lipid content in Bruch’s membrane throughout life is higher in the macula compared to the periphery [40]. The age-related exponential decline in hydraulic conductivity of Bruch’s membrane is also more marked in the macula [41]. Accumulation of esterified cholesterol renders the Bruch’s membrane hydrophobic, impairing diffusion between the RPE and the choriocapillaris and disrupting photoreceptor functions [42].

Because the activity of lysosomal activity of RPE decreases with age, abnormal materials collect as in the inner portion of Bruch’s membrane between the basement membrane of the RPE and the inner collagenous layer: the drusen. Drusen are believed to play a major role in the induction of RPE detachment. The clinical hallmark of AMD is the appearance of drusen. The presence of drusen is a strong risk factor for the development of both atrophic and exsudative forms of AMD (For more details, see section III-3).

No or very few glycation endproducts (AGEs) or receptor for AGE (RAGE) were found in photoreceptors or RPE in normal retina. In contrast, AGE and RAGE were identified in RPE and photoreceptors in early AMD and GA [43]. AGEs have been identified in RPE in association with drusen [44] and lipofuscin [45]. AGE accumulate within Bruch’s membrane and may therefore alter RPE-Bruch’s membrane interaction and RPE function [46]. AGEs decrease the hydraulic conductivity of Bruch’s membrane [47]. They decrease RPE lysosomal enzyme function and influence the RPE phenotype [48]. In addition, AGEs modify lipids, proteins and nucleic acids, which are elevated in AMD [49] and leads to the upregulation of genes that promote aging in RPE cell cultures [50]. Because AGEs also increase the expression of the potent angiogenic factor, VEGF (vascular endothelial growth factor), in RPE cells, they may participate to the development of exsudative AMD, by stimulating the proliferation of vascular endothelial cells of the choroid [51].
The insulin growth factor-1 (IGF-1) is also produced by RPE cells and is expressed in surgically excised membranes of CNV [52]. IGF-1 stimulates the proliferation of cultured cells from CNV tissue, suggesting that it may participate to neovascularization during exsudative AMD [52]. Because IGF-1 stimulates the expression of VEGF, its effects on CNV may be indirect. RPE cells express the receptor type 2 of somatostatin (sst2). Interestingly, somatostatin have been recently shown to inhibit IGF-1 mediated induction of VEGF in RPE cells [53], suggesting that the use of somatostatin or its analogues may be a therapeutic option to fight CNV in AMD. ROS also induced an increase in VEGF and in the angiogenic factors FGF2 (fibroblast growth factor 2), and a decrease in the anti-angiogenic factor PEDF by RPE cells [54-56]. In addition, aged RPE cells express less PEDF. Altogether, these studies strongly suggest that AGEs and ROS induce a shift toward the induction of angiogenic factors to induce neovascularization in the choroid.

Age-related hemodynamic changes have been observed in Bruch’s membrane in addition to hydrodynamic changes. There are evidences that the choroidal blood flow is impaired in aged individuals. The age-related decrease in the foveal choriocapillaris blood flow is further attenuated in AMD patients [57]. Lipoidal infiltration of the sclera and Bruch’s membrane leads to an increased resistance of the sclera and choroidal vessels, resulting in decreased choroidal perfusion and impaired RPE transport. Altogether, these changes of the choroidal hemodynamic properties lead to lipid infiltration of Bruch’s membrane, formation of drusen and RPE atrophy.

3. Changes in the inner part of the retina

Another characteristic of retina aging is the decrease in the number of ganglion cells in both the fovea and peripheral retina [10,58]. Activation of astrocytes has been also detected in aged human retina, with the expression of glial fibrillary acidic protein (GFAP) [59].

4. Aging of retina and oxidative stress

Aging has been defined as “the progressive accumulation of changes with time that are associated with or responsible for the ever-increasing susceptibility to disease and death which accompanies advancing age” [60]. Among the numerous theories of aging, the free radical theory of aging is of particular interest for the retina. The free radical theory of aging proposes that aging and age-related disorders are the result of cumulative damage arising from reaction involving reactive oxygen species (ROS) and oxidative damage. ROS include the singlet oxygen (\(^{1}\text{O}_2\)), the hydrogen peroxide (\(\text{H}_2\text{O}_2\)), superoxide anion (\(\text{O}_2^-\)), the hydroxyl free radical (\(\text{OH}^-\)), the hydroperoxyl radical (\(\text{HO}_2^-\)) and the lipid peroxyl radicals. Carbohydrates, membrane lipids proteins and
nucleic acids are all vulnerable to ROS. Age-related oxidative damage has been shown to contribute to lipofuscin genesis, a hallmark of AMD.

4a. Oxidative stress in the retina
The retina is an ideal environment for the generation of ROS: 1- oxygen consumption by the retina is much greater than by any other tissue [61], 2- the retina is subject to high levels of cumulative irradiation, 3- membranes of the photoreceptor outer segments are rich in polyunsaturated fatty acids (PUFAs) [62], 4- the neural retina and the RPE contain an abundance of photosensitizers and 5- the phagocytosis by the RPE is itself an oxidative stress and results in the generation of ROS [63].

4b. Antioxidant defense in the retina
To minimize the destructive potential of ROS, cells have developed a number of defenses mechanisms. Two main types of antioxidant defense system exist: an enzymatic system and a non-enzymatic system. Among the enzymes involved in the antioxidant defense are the superoxide dismutases (SODs), catalase, glutathione peroxidase (glutathione-Px), heme oxygenase and phospholipases. SOD catalyzes the quenching of the superoxide anion to produce hydrogen peroxide (H$_2$O$_2$) and oxygen. This is the first step metabolic defense against cellular oxidative stress. Two SODs exist, the manganese (Mn)-SODs or SOD2 and the copper and zinc (CuZn)-SODs. Catalase is an iron (Fe)-dependent enzyme that scavenges H$_2$O$_2$ either catalytically or peroxidatively. Catalase, which is highly expressed in RPE, converts hydrogen peroxide in water and oxygen. Glutathione-Px uses glutathione as an electron donor to reduce organic hydroperoxide. It is dependent on selenium as a cofactor. Vitamin E, A and C, and glutathione (GSH) belongs to the non-enzymatic defense. GSH is a tripeptide which scavenges oxidizing agents by reaction with them. RPE which possesses these two antioxidant defenses also contains melanin which is an ROS scavenger. Photoreceptors also content the antioxidant enzymatic defenses. But, it has developed a very powerful antioxidant system, the continuous replacement of their cellular constituent (Marshall, 1985).

III. Age-related macular degeneration
1. Functional and structural alterations of the retina
Human retina is subjected to a great number of different degenerative diseases which target the photoreceptor-RPE-choriocapillaris complex. Among these diseases the age-related macular degeneration (AMD) affects the macula. AMD is the leading cause of irreversible blindness in developed country for those over the age of 65 years and about 35% of the human population of 75
years or older has some degree of AMD [64-66]. It currently affects around 12.7 million people in Europe and north America [67,68]. Because the life span of humans continues to increase as a function of improved nutrition and increased awareness of environmental factors, AMD cases are expected to increase twice in the next 25 years [69].

Functional studies support the histological evidence for preferential vulnerability of rods in aging and AMD. In old adults in good retinal health there is a reduced rod-mediated light sensitivity, the magnitude of which is similar throughout the parafoveal area. Scotopic sensitivity is significantly lower in early AMD patients than in aged-matched controls without AMD [70]. Consistent with the pattern of scotopic sensitivity loss, delays in rods-mediated dark adaptation are greater than those for cone-mediated dark adaptation in AMD. Topographic analysis of photoreceptor dysfunction and loss reveals that the disease process in AMD is spatially heterogeneous across the macula: the scotopic sensitivity loss and the loss of rods in early AMD patients is greatest near the fovea center and declines markedly to the edge of the macula [14,71]. In contrast, photopic sensitivity and loss of cones changes are not important over the same distance [70].

In dry AMD which is the most common form (90%), there is progressive atrophy and degeneration of the RPE with subsequent loss of choriocapillaris and photoreceptors within the macula. The perifoveal rods degenerate first and rod loss is generally greater than cone loss at the same location [14,71]. Most patients with dry AMD notice a slow, progressive decline in central vision. Geographic atrophy (GA) of RPE is a form of advanced AMD that is responsible for both moderate and severe central vision loss. GA refers to confluent areas (defined as 175 µm in minimum diameter) of RPE cell death accompanied by overlying photoreceptor atrophy [72]. More generally, GA could be definite as cell death at the level of the RPE, outer neural retina and choriocapillaris. GA is responsible for 20% of the legal blindness from AMD [73]. It is the natural end stage of the atrophic AMD process when choroidal (or subretinal) neovascularization (CNV) does not develop [74]. In contrast, patients with exsudative AMD usually recognize a sudden loss of vision from either bleeding or fluid leakage from abnormal vessels that have grown from the choriocapillaris beneath the RPE and macula: CNV. The exsudative AMD, although representing only around 10% is responsible for about 80% of the legal blindness from AMD [73]. For most of AMD patients there is no effective treatment.

GA often develops first in the region near the fovea, but does not involve the center of the fovea. It progresses gradually over time, sparing the fovea until late in the course of the disease [74]. RPE alteration is generally first observed in small areas which tend to enlarge and coalesce over time, leading to a horseshoe of atrophy surrounding, but not involving the foveal center.
Finally, the fovea becomes atrophic. The mean rate of enlargement over a two year period was 2.2 MPS disc areas (equivalent to 5.6 mm² on the retina) and increases to about five MPS disc areas, after which the rate plateaus GA is bilateral in more than half of patients [74,75] and may also develop following the flattening of a RPE detachment [76-78]. GA is associated with deposits in and thickening of the Bruch’s membrane [74].

Photoreceptors that overlie both hard and soft drusen (focal deposits of extracellular materials lying between the basal lamina of the RPE and the inner collagenous layer of the Bruch’s membrane, a hallmark of AMD) are also greatly affected. This includes deflection and shortening of rod inner and outer segments [79]. When drusen increase in size, loss of rod outer segment and changes in synaptic cytoarchitecture over large drusen are observed. Cones display similar structural changes, as well as decrease in cone opsin expression [79]. GA leads to gradual progression of visual loss, most likely because photoreceptors overlying areas of RPE degeneration are metabolically dependent on RPE cells.

CNV is a major cause of visual loss in AMD. The pathogenesis of CNV in AMD is poorly understood. Newly formed vessels, originating from choroidal vessels break through the Bruch’s membrane into the subretinal pigment epithelium space. Hypoxia, an important feature of retinal neovascularization, may play a role in the development of CNV, as well. Diffusion of oxygen from the choroid to the RPE and retina decreased due to age-related thickening of Bruch’s membrane with lipophilic material [40,80]. Alterations of the Bruch’s membrane are also observed in AMD. Among the major alterations are the deposition of oxidized lipids in [81] and an increase presence of macrophages [82]. It has been proposed that macrophage recruitment into the subretinal space removes the deposit of cholesterol esters and oxidized lipids, which results from the phagocytosis of photoreceptor outer segments [83]. However age-related accumulation of the oxidized lipoproteins may alter the behavior of the attracted macrophages, resulting in the upregulation and secretion of matrix metalloproteinases and glycosidases [84]. Then, digestion of the collagen network of Bruch’s membrane would lead to the development of CNV. These processes participate at the inflammatory theory in AMD.

Moreover, different studies suggest an important role of the potent angiogenic factor, VEGF, in CNV. VEGF has been identified in RPE of choroidal neovascular membranes [85,86]. It has been also detected at a higher level in RPE of macula from patients with age-related maculopathy [87] and in patients with AMD and CNV [88], in comparison to healthy controls. RPE cells upregulate VEGF secretion to their basal side under hypoxia [89,90]. Therefore, a paracrine effect of VEGF, secreted at the basolateral face of RPE toward the choriocapillaris, which express the VEGF receptors, may explain CNV in AMD.
In addition, and apparently more surprisingly, a large number of histopathological studies examining the retinas of eyes diagnosed with AMD have identified numerous structural and molecular abnormalities in retinal cells that overlie drusen. Increases in the intermediate filament expression, including vimentin and glial fibrillary acidic protein (GFAP), in Müller glial cells and astrocytes have been observed [91,92]. Moreover, alteration in the inner nuclear layer has been observed in both atrophic and exsudative AMD, with apoptotic amacrine cells [93].

2. Risk factors

Large epidemiological studies have provided factors associated with an altered risk of AMD, including demographic and cardiovascular factors, light exposure, dietary and medication factors. The first factor of risk is age, since incidence, prevalence and progression of all forms of AMD increase with age [68,94,95]. All forms of AMD are more often seen in white population [96-98]. There is no association between iris color and AMD [95]. Female have a slightly greater prevalence of AMD, especially for the exsudative form [99]. A strong relationship exists between coronary artery disease and atrophic forms of AMD [100]. Arterial hypertension, coronary diseases and smoking have been also associated with an increased risk of developing AMD [100]. Lens opacity and previous cataract surgery are also risk factors for AMD, highlighting the role of excessive light in retinal damage [100]. Both prior and current smoking is at increased risk for AMD [101,102]. This may be due to the smoking’s effects on antioxidant metabolism and choroidal blood flow, and/or to the angiogenic effects of nicotine. Total fat intake and especially high linolenic acid intake was associated with increased risk of AMD, whereas high docosahexaenoic acid intake was associated with a reduced risk of AMD [103,104]. Moreover, data from the AREDS show that supplementation with very high doses of vitamin C, vitamin E, β carotene and zinc provide a protective effect on progression of advanced exsudative AMD in patients with extensive drusen [105], suggesting a role of oxidative stress in the etiology of AMD.

Twin-concordance, linkage studies and biomolecular investigations implicate genetic factors in AMD. In addition, several inherited macular dystrophies share many important clinical and histopathological similarities with AMD, including, abnormal accumulation of intracellular pigments, such as lipofuscin in the RPE, loss of function and/or degeneration of photoreceptors and loss of central vision [106-108]. To date, however, none of the genes implicated in monogenic inherited macular dystrophies have been found to have a significant role in the genetic predisposition to AMD or to confer increased risk of AMD.

In contrast, it has been recently reported that a variation in the factor H gene (*HF1/CFH*) dramatically increases the risk for developing AMD
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[109-114]. CFH encodes a major inhibitor of the alternative complement pathway, accumulated within drusen and is synthesized by the RPE. DNA sequencing of CFH revealed a common coding variant, Y402H, that significantly increases the risk for AMD and may account for approximately 50% of AMD in older adults. In addition, this CFH polymorphism is a major risk factor of soft drusen [115]. The tyrosine-histidine mutation at amino acid 402 is in a region of CFH that binds C3b, heparin, sialic acid and C-reactive protein. Thus, this single nucleotide polymorphism might affect different CFH functions, including attenuated complement inhibitory function. One hypothesis is that CFH variant associated with AMD may put RPE and choroid cells at sustained risk for complement attack. It appears likely that the inheritance of at-risk CFH haplotype, in combination with an infectious agent or other activators of the alternative pathway, such as immune complexes, amyloid-β peptide, or cholesterol, may substantially increase susceptibility to AMD. In addition, these data strongly suggest that molecules involved in complement activation and its regulation may become prime targets for therapeutic intervention in AMD.

Contrary to CFH, the apoE ε4 allele is associated with a decreased risk of exsudative AMD, while the ε2 allele is associated with an increased risk [116,117]. Finally, the existence of large amounts of soft drusen in the macular area is believed to be a major risk factor for AMD. In addition, four independent risk factors for the development of CNV have been established: The presence of five or more drusen, focal RPE hyperpigmentation, systemic hypertension, one or more large drusen and extramacular drusen [118,119].

3. Drusen

3a. Drusen, a hallmark of AMD

The clinical hallmark of AMD is the appearance of drusen. Drusen are focal deposits of extracellular materials lying between the basal lamina of the RPE and the inner collagenous layer of the Bruch’s membrane. Clinically, drusen are classified morphologically either as hard or soft [120-122]. The size, number and extent of confluence of drusen are important determinants for the risk of developing AMD [123]. Indeed, in small number, they are not considered risk factors for the development of AMD [124], but numerous hard drusen are an independent risk factor for visual loss from AMD [118]. Typically, drusen are clustered in the central macula.

Despite the numerous classification systems employing different criteria, there is a general consensus about several characteristics of age-related drusen. Hard drusen are defined as small, punctuate and yellow nodules (less than 63 μm in diameter), whereas soft drusen tend to be larger than hard forms [125-127]. In advanced stage of AMD, hard drusen can coalesce to form confluent plaques that are associated with the atrophy of large patches of RPE and
macular regions of retina. It has been observed that when hard drusen disappear, the overlying RPE and outer neural retina may become atrophic leading to GA. The presence of soft, large and/or confluent drusen is correlated to the occurrence of CNV [126]. It has been proposed that there is a relationship between drusen deposition and the choriocapillaris [128]. More recently, it has been shown an association of drusen deposition with choroidal intercapillary pillars in the aging human eye [129]. Although drusen were first described nearly 150 years ago, the cellular and molecular events involved in their formation have not been fully elucidated.

3b. Composition of drusen and origin of the drusen biogenesis

Interestingly, many of the molecules which compose drusen are common the pathological deposits associated with other neurodegenerative diseases including Alzheimer’s disease (AD), raising the possibility that common pathogenic pathways may be involved in their formation. To date, no significant differences in the molecular composition of hard and soft drusen have been identified, suggesting that they may have a common origin [124].

Although numerous studies provided evidence that drusen contain lipids, including both esterified and unesterified cholesterol [40,42,130,131], the exact origin of drusen-associated lipids (from cellular membranes or the vasculature or a combination of both sources) remains unelucidated. Lipoprotein components of drusen such as apolipoprotein E (ApoE) are transcribed locally, whereas other molecules such as amyloid P appear to have a hepatic origin. Glycoconjugates such as N-acetylglucosamine, sialic acid and galactosamine disaccharide are identified as major drusen carbohydrates, this latter confined to the central core domain within drusen [132,133]. Numerous proteins are identified within drusen. Among them are ubiquitin, integrins, tissue inhibitor metalloproteinase 3 (TIMP 3), advanced glycation endproducts (AGE), beta-amyloid, amyloid P, ApoE, factor X, the extracellular matrix components, fibronectin, vitronectin, immunoglobulin light chains, components of the complement cascade including, the membrane attack complex, C5b-9, the activated complement components C1q, C3c, C3d, C4, C5 and C9, and the MHC class II antigens [134]. A large number of these proteins are synthesized by the RPE. Cellular materials residing within drusen have also an RPE origin. RPE constituents, such as lipofuscin and melanin are observed within small early drusen, supporting the hypothesis that RPE participates to their formation. Cell-associated molecules such as HLA-DR and specific CD antigens (CD1a, CD83 and CD86) are also detected within drusen [135].

Many theories for drusen biogenesis have been advanced. They involve RPE transformation, vascular deposition and leukocyte migration and activation. However, they are highly speculative. For example, although different studies described the appearance of RPE debris blebbing into drusen and the presence of lipofuscin and melanin granules within drusen, it is unclear...
if drusen are a cause or a consequence of RPE dysfunction, leading to RPE cell death. Does accumulation of drusen interfere with the RPE homeostasis, leading to RPE degeneration, or do drusen develop as a consequence of RPE dysfunction?

One of the best documented hypothesis which may explain the cellular and molecular mechanisms involved in drusen biogenesis and perhaps the etiology of AMD is the inflammatory and/or immune-mediated process, including the recruitment and maturation of dendritic cells. The argument is that many drusen-associated components are actors of inflammatory and/or immune (C5b-9, immunoglobulin and MHC-II antigens) processes [134]. Moreover, the presence dendritic, antigen-presenting cells, observed in AMD suggests a role for immunological and inflammatory processes in drusen pathogenesis [134]. It has been proposed that choroidal dendritic cells are activated and recruited by locally damage and/or sublethally injured RPE cells. In this model, RPE cells would serve as the source of cytokines and inflammatory factors that initiate dendritic cell recruitment and activation. The authors proposed that drusen development would occur in at least two distinct stages: a nucleation stage, in which RPE debris and choroidal dendritic cell-derived materials accumulates in the sub-RPE space, and a maturation stage, in which drusen-associated constituents are deposited around the core. The addition of RPE cell debris or proteins secreted in response to the presence of dendritic cells would increase the drusen mass. This type of mechanism would account for the difference in the composition of drusen between the core and surrounding materials [134]. The presence of anti-RPE autoantibodies in the sera of patients with AMD may be explained by the activation of dendritic cells which initiate an autoimmune response to RPE antigen [134]. Moreover, an in vitro study on the ARPE-19 cell line, shows that RPE cells over express the monocyte chemotactic protein-1 (MCP-1) (also known as Ccl-2) in response to wound healing, confirming the participation of RPE in receptor-ligand interaction for macrophage recruitment and/or dendritic migration [136]. More recently, an animal model confirms the role of the immune system in the development of AMD [83]. Knock-out mice with a null phenotype that are deficient for MCP-1 and its cognate C-C chemokine receptor, Ccr-2, develop the morphological and ultrastructural features of AMD, including, drusen and the deposition of lipofuscin in the RPE.

3c. Roles of drusen in RPE and photoreceptor cell death

Little is known concerning the exact effects of drusen on the retina, or why they are significant risk factor for vision loss. Drusen affect various aspects of vision prior to loss of visual acuity. They change color contrast sensitivity, central visual field sensitivity, macular recovery function and spatiotemporal contrast sensitivity [134,137,138].
It has been shown that drusen cause lateral stretching of the RPE monolayer and physical displacement of the RPE from the choriocapillaris. This drusen-induced displacement creates a physical barrier that block the diffusion of nutrients and regulatory molecules necessary for the maintenance of the health of the RPE and photoreceptors. The accumulation of debris between RPE and the Bruch’s membrane is seen early during the development of AMD.

Drusen contain many components of the complement cascade, including MCP-1. Ambati et coll. postulate that because of impairment of macrophage recruitment in the MCP-1−/− and Ccr-2−/− mice, there is the accumulation of complement fragments, which may damage RPE [83]. Apoptosis is the major cell death process of RPE in AMD (For more details, see section IV-3). Cells undergoing apoptosis do not typically recruit leukocytes, such as dendritic cells. Therefore, if these data do not rule out a major role of the inflammatory and/or immune-mediated in the process of drusen biogenesis, they are inconsistent with a direct role in the process of RPE cell death. But, the immune system may participate to the development of exsudative AMD, because MCP-1-Ccr-2 pathway activates macrophages which may contribute to the degradation of the Bruch’s membrane and stimulation of CNV, through the induction of the angiogenic factor, VEGF. Then, Bruch’s membrane destruction may lead to RPE cell death.

Cellular and molecular changes observed in photoreceptors overlying drusen are progressive. Shortening of the inner and outer segments of both rods and cones, redistribution of rod opsin and decrease in cone opsin expression, and the loss of synapse-related machinery compromise the function of photoreceptors, leading to gradual visual loss. Finally, photoreceptors die by apoptosis [93]. The clustering of apoptotic photoreceptors at the edges of atrophy is consistent with the pattern of cell death and expanding GA in which RPE cell die first, following by photoreceptor death due to loss of RPE support function [74]. In addition, photoreceptors in AMD eyes labeled with anti-Fas antibodies, whereas Fas was nearly undetectable in photoreceptors of normal eyes [93]. Interestingly, plasma soluble Fas ligand (FasL) is increased in patients with AMD compared with normal eyes [139]. Altogether, these studies strongly suggest that Fas/FasL may trigger the initiation of photoreceptor apoptosis in AMD.

Several explanations for the drusen-associated photoreceptor degeneration are advanced. Because viable RPE is a prerequisite for normal photoreceptor function and maintenance of the retinal microenvironment, it is also possible that drusen affect indirectly photoreceptor viability by compromising the photoreceptor-supply function of RPE. Indeed, RPE is necessary for many functions of photoreceptors: exchange of metabolites, regeneration of photoreceptor pigments, structural support of outer segments and phagocytosis.
of discs. A second hypothesis is that defects in the synaptic architecture in the outer plexiform layer, displacement of photoreceptors and disorganization of their outer segments by drusen may damage the structural integrity of photoreceptors, leading to render them dysfunctional and cause cell death. This hypothesis is confirmed by the fact that compromise in the structural integrity of outer segment in the inherited retinal degeneration slow (RDS) mouse have been demonstrated to lead to apoptosis of photoreceptors [140]. Third, it is possible that drusen may impair the normal exchange of ions, metabolites and nutrients between the choroidal blood supply and photoreceptors, by establishing a physical barrier to diffusion. Impairment of oxygen diffusion from the choroidal blood supply to the retina has been shown to result to photoreceptor cell death in a retinal detachment animal model; this is consistent with the hypothesis of the impairment of diffusional barrier [141]. Finally, it is also possible that drusen contain cytotoxic molecules for RPE and/or photoreceptors, including photo-oxidative derived products, ApoE and amyloid beta. Studies have shown that intravitreal injection of amyloid beta induces photoreceptor cell death and Müller cell hypertrophy [142,143].

IV. Etiology of AMD

Many hypotheses have been proposed to explain RPE degeneration and AMD, including gene mutations, light damage, oxidative stress, or lipofuscin accumulation. Theses hypotheses are not exclusive.

1. Genetic causes

AMD may have a significant genetic component to its etiology. The difficulties in the characterization of potential involvement of genes in AMD come from the late onset of the pathology. Parents of affected individuals are often. Several inherited macular dystrophies share many important clinical and histopathological similarities with AMD, including, abnormal accumulation of intracellular pigments, such as lipofuscin in the RPE, loss of function and/or degeneration of photoreceptors and loss of central vision [106-108]. To date, none of the genes implicated in monogenic inherited macular dystrophies have been found to have a significant role in the genetic predisposition to AMD or to confer increased risk of AMD. Whereas no specific genetic causes of AMD are known, genetics defects responsible for a number of monogenic forms of early macular degeneration has been identified, each with some features in common with AMD including, abnormal accumulation of intracellular pigments, loss of function and/or degeneration of photoreceptors and loss of central vision [106-108]. Causative roles of mutations in the TIMP3, VMD2, ELOVL4 in AMD could not be demonstrated by genetic studies [144-147]. The role of ABCA4, that encodes a transmembrane protein located in the disc of rod
and foveal cone outer segments that is involved in ATP dependent transport of retinoids from photoreceptors to RPE, is questionable [148-150]. Mutations in $ABCA4$ has been implicated in Stargardt disease, retinitis pigmentosa and cone-rod dystrophy [106]. Failure in ATP dependent transport of retinoids results in deposition of lipofuscin in the RPE [150], which may be deleterious to the RPE with consequent secondary photoreceptor degeneration. Therefore, it has been proposed that certain missense mutations of $ABCA4$, present in heterozygous form may increase susceptibility for AMD [107]. But, this hypothesis has been recently questioned [151,152].

2. Light damage

Compelling evidences implicating light damage in the pathogenesis of AMD have been collected over the last three decades. Although, it has been established that ultraviolet (UV) and visible light have the potential to induce irreversible damages in the retina [153], cornea and lens protect retina from short-wavelength radiation by absorbing radiation below 295 nm and below 400 nm, respectively in human adults. Therefore, it is the blue light (442 nm) which is toxic for the retina. The power required to cause photic damage is about one log unit lower for blue light than for infrared (IR) radiation (1064 nm) [154]. It has been shown that the potential for blue light toxicity increases nine fold over the first and the ninth decade of life [155]. Two distinct retinal layers are susceptible to photochemical injury, the photoreceptors and the RPE. It has been shown that short exposure of relative intense short-wavelength can induce RPE damage in primates [154]. Because, the light damage is dependent on oxygen concentration and that antioxidants reduce the light damage, it has been proposed that blue light damage has an oxidative origin [156-158]. Two of the main effects of light is a reduction of the long-chain of PUFAs (mainly $22:6\omega3$) contained in the membranes of the photoreceptor outer segments and RPE, and an increase in the levels of lipid conjugated dienes, providing evidence that lipid peroxidation plays a major role in retinal light damage.

Chromophores, such as lipofuscin, cytochrome c oxidase, and melanin have been proposed to mediate the blue light damage to retina. Photochemical reactions involve a reaction between an absorbing molecule and energetic photons. This reaction leads to the production of ROS in the presence of oxygen. ROS which include singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radicals are highly toxic and can induce lipid peroxidation, protein oxidation and DNA damage, including mitochondrial DNA.

Short exposure of intense blue light induce RPE damage, whereas, long exposure to moderate intensity of light produce damage at the level of photoreceptors. It is to note that cones are more vulnerable than rods [159]. It has been suggested that this is due to a difference in repair capacity between the two types of photoreceptors since rod outer segments are replaced more
rapidly than cone outer segments [160]. The power required to cause photic damage was 70 to 1000 times lower for blue light than for the infrared wavelength.

Therefore, a number of investigators have proposed the use of sunglasses which attenuate short-wavelength light to reduce phototoxic potential of light. Yellow/orange filters would reduce the in vivo aerobic photoreactivity of lipofuscin by approximately 90%. In addition, an in vitro study, using yellow intraocular lenses show that theses filters would reduce by 80% cell death of RPE cells cultured in the presence of lipofuscin and exposed to light [161].

3. Oxidative stress

Among the environmental factors that could affect retinal structure and functions, genomic damage associated with oxidative stress appears to be an important contributor of AMD.

3a. Mechanisms of oxidative stress-induced RPE cell death

The antioxidant capacity of RPE decreases with age. Catalase activity in both macular and peripheral RPE is negatively correlated with donor age, while the superoxide dismutase activity remains unchanged [162]. There is also a tendency of lower total plasma GSH in the AMD patient in comparison to controls (young-age matched non AMD individuals) [163]. Along with the physiological oxidative stress, environmental factors, such as smoking and intense light exposure increase the risk for RPE degeneration [164,165].

Study on cell culture is the major approach for mechanistic studies of the role of oxidative stress and antioxidant defense systems in RPE degeneration. Among oxidants, hydrogen peroxide (H₂O₂), 4-hydroxynonenal (HNE) and t-butyl-hydroperoxide (t-BHP) are widely used to investigate oxidative in vitro stress-induced RPE cell injury. Different studies showed that H₂O₂ induced RPE cell apoptosis through an oxidative stress. It has been shown that (50-200 mM) H₂O₂ induces RPE cell apoptosis with an increased caspase-3 activity [166]. RPE cell apoptosis is accompanied by a decrease in the expression of the antiapoptotic protein, Bcl-2 and an increase in the expression of p21 and p53. Cleavage of caspase-3 in hydrogen peroxide-exposed RPE cell death is confirmed by the study of Kim et Coll. [167]. These authors show that moderate concentrations of H₂O₂ (400-600 µM) induced RPE cell death through an apoptotic mechanisms with cleavage of poly (ADP-ribose) polymerase (PARP), whereas higher H₂O₂ concentrations induced RPE cell necrosis [167]. RPE cell treatment with sublethal doses of hydrogen peroxide induces a disruption of the junction and barrier integrity visualized by paracellular flux across the RPE monolayer [168], suggesting that oxidative stress may contribute to the pathogenesis of AMD, through disruption of the blood-retinal barrier. This is accompanied of cytosol β-catenin increased and
higher steady state levels of heat shock protein (Hsp) 27 and Hsp70. In addition, it has been shown that H$_2$O$_2$-mediated sublethal oxidative stress inhibits the outwardly rectifying chloride current [169]. This study indicates a possible role of the oxidative stress-induced modulation of CIC chloride channels in the disruption of RPE chloride transport, which may result in the alteration of fluid transport in the subretinal space and alteration of RPE. In addition, t-BHP suppresses voltage-dependent (K+) current I(K) in RPE cells [170].

T-BHP differs from lipid hydroperoxides in being water-soluble. Unlike hydrogen peroxide, it is not metabolized by peroxide actions of catalase. It is principally inactivated by direct and glutathione transferase-promoted reduction of GSH [171]. T-BHP is a relative stable hydroperoxide and penetrates cell membranes readily. Once it is within the cell, it induces oxidative stress by 2-electron oxidation and by metal ion and metalloprotein-catalyzed free radical processes. It is detoxified by reduction to t-butanol by GSH peroxidase and is often associated with GSH depletion. T-BHP induces a dose-dependent RPE cell death [172,173]. At a high concentration, t-BHP (900 µM) rapidly induces RPE cell death (within 2 hours) with cell-swelling. RPE cell membranes become permeable to the vital dye trypan blue, indicating that cells die by a process involving an acute necrosis [173]. In contrast with lower concentrations of t-BHP (300 and 500 µM), RPE cell die with a slower kinetics, with characteristics supposed to be specific of apoptosis: cell shrinkage, nuclear chromatin condensation and caspase activation [172]. All these events occur before the loss of membrane integrity. It is interesting to note that t-BHP-induced RPE cell apoptosis does not show typical DNA fragmentation with size interval fragments of 180-200 base pair, in these conditions. However, RPE cell nuclei were positive for TUNEL staining, suggesting that larger fragments of DNA may occur after exposure to low concentrations of t-BHP [163]. The absence of 200 bp laddering in RPE cells exposed to lethal oxidative stress (menadione) has been confirmed by the study of Zhang et Coll. [174]. In addition, these authors show that menadione-induced RPE cell death was not accompanied by the cleavage of caspase-3 and -9. In contrast, menadione induces mitochondrial membrane depolarization, release of cytochrome c, nuclear translocation of the apoptosis-inducing factor (AIF) and subsequent 500 bp laddering, and nuclear shrinkage [174]. These findings highlight the complex nature of RPE cell death processes which may depend on the type of oxidative stress.

Some evidences show that oxidative stress-induced RPE cell apoptosis involves a mitochondria-generated signaling mechanism. An early decrease of the mitochondrial membrane potential is detected in t-BHP-exposed RPE cells. This event occurs before activation of caspase 3 and DNA fragmentation [172], suggesting that mitochondria is a primary target of oxidative stress in
RPE cells. It has been proposed that the mitochondrial permeability transition (MPT) may be a phenomenon by which mitochondria sense the initial oxidative stress in RPE cells [163]. This was recently confirmed by Hinton et Coll., which showed that ceramide-induced RPE cell apoptosis is accompanied by an increase in MPT, which could be inhibited by the hepatocyte growth factor (HGF) [175]. Moreover, in RPE cells, mitochondrial DNA (mtDNA) is particularly prone to oxidative damage in comparison to nuclear DNA [176].

Sphingomyelin is preferentially concentrated on the plasma membrane and its hydrolysis generates ceramide. Ceramide has been implicated in a variety of cellular process, such as apoptosis and senescence and in ROS-mediated RPE cell death. RPE cell treatment with either H$_2$O$_2$ or t-BHP results in the intracellular production of ceramide [177]. These authors showed that addition of C2 and C6 synthetic ceramides cause apoptosis in RPE cells, whereas cell treatment with the immediate precursor of ceramide, dihydroceramide does not. More recently it has been shown that RPE cells treated with C2 ceramide undergo apoptosis with a caspase-3 activation and a decreased catalase enzymatic activity [175]. These two studies suggest that ceramide may play a role of in ROS-mediated RPE cell degeneration in AMD.

In conclusion, many in vitro studies showed that oxidative stress-induced lethal effects on RPE cells involves both necrosis and apoptosis, the choice of the cell death feature depending on the strength of the stress more than the type of the stress. But, it is to note that the indicators of apoptosis used in these studies, such as TUNEL reaction, formation of DNA ladders, nuclear condensation, or phosphatidylserine exposure are extremely late in the cell death process. Therefore, it is difficult to be sure that what the investigators identify as apoptosis in in vitro studies reflects the cell death process during oxidative stress-induced RPE degeneration during AMD. The complexity of the nature of RPE cell death processes is confirmed by the recent study of Brossas et coll. These authors showed that ethanol-mediated oxidative stress that induced lethal RPE cell death, occurs with DNA fragmentation exhibiting a sub-G1 peak, suggesting necrotic cells [178]. More surprisingly, PARP cleavage and activation of a caspase-independent LEI/DNase II pathway in the ethanol-exposed RPE cells suggests apoptosis [178]. Therefore, this study shows that an oxidative stress may induce cell death with characteristics of both apoptosis and necrosis. It remains to elucidate whether ethanol induce RPE cell death via two independent cell death pathways, apoptosis and necrosis, or whether it is an unique cell death pathway which possess the features of both necrosis and apoptosis.

It is important to note that the presence of a caspase does not prove that cell death is caspase dependent and caspase activation does not always mean apoptosis or even cell death [179]. Although, caspase activation has been observed in many studies describing the lethal effects of oxidative stress on
RPE cells, very few demonstrate the role of caspase activation during oxidative stress-induced RPE cells, for example by down regulating the expression of caspase or using specific inhibitory substrate of caspase. Conversely, a lack of phenotype resulting from knockout of a caspase, or caspase regulator does not eliminate the possibility of compensation by other caspase. Proteolytic damage by any several proteases, not uniquely caspases, can trigger cell death. For example, activation of lysosomal proteases, granzyme B and MMPs may participate to cell death pathways. But, although PARP may be highly destructive in the absence of caspase activation, its activation is not necessarily synonymous with cell death.

To better understand the different molecular mechanisms involved in RPE cell death after different type of oxidative injury, the group of Hjelmeland examined the regulation of genes differentially expressed in RPE cells submitted to lethal oxidative stresses induced by t-BHP, H\textsubscript{2}O\textsubscript{2} and HNE [180,181]. As expected, a common response is observed that includes several genes differentially regulated by all three oxidants, including apoptosis, cell cycle regulation, signal transduction, transcriptional regulation and cell-cell communication [180]. But, a large number of genes were also differentially regulated by one oxidant only. In addition, it has been observed that oxidative stress in differentiated RPE cells alters the expression of genes toward a level characteristic of undifferentiated state [182]. These studies raise the question regarding the generality of data that involve the use of one single oxidant.

3b. Role of the pigments in retinal degeneration and AMD

Both RPE cells and photoreceptors are the targets of the photochemical injury. Cell damage is likely to be mediated by lipofuscin and the visuals pigments. Blue light is a source of oxidative stress via its interaction with retinal chromophores. RPE is a closed system, with RPE cells accumulating ROS damage without the possibility of renewal, whereas photoreceptor is more likely an open system with an abundant component renewal. Therefore, it has been hypothesized that lipofuscin is of particular importance in the photochemical injury of the retina in AMD.

Lipofuscin is a prominent autofluorescent age-pigment. Increased lipofuscin accumulation has been associated with various retinal degenerations including Leber’s amaurosis, Best’s disease and Stargardt’s disease. More than 90% of the lipofuscin amassed in RPE cells originates from conjugates formed by visual cycle retinoids in photoreceptors, which is then deposited in RPE cells subsequent to outer segment disc phagocytosis. Lipofuscin is thought to represent irreducible end-products of outer segment breakdown in RPE, that accumulate with age [183]. Visible autofluorescence due to lipofuscin follows closely the normal distribution of rods, which is low in the foveal center and high at 2-4 mm [184]. A recent proteome analysis reported the presence of
malondialdehyde, 4-hydroxynonenal (HNE) and AGEs in RPE lipofuscin [45]. In addition, one of the first identified and structurally characterized constituent of lipofuscin, A2E, which is a Schiff base reaction product derived from a molecule of vitamin A aldehyde and one molecule of ethanolamine [185] can photogenerate A2E epoxide [186]. Altogether these data suggest that lipofuscin is made up of oxidatively modified proteins. Moreover it has been shown that lipofuscin have the capacity to generate ROS because 1- Isolated lipofuscin granules exposed to white light generate superoxide anions [187], 2- ROS production due to lipofuscin, measured by oxygen uptake, in RPE cells increases with age [188], 3- Exposure to blue light (400-550 nm) results in the photogeneration of singlet oxygen, superoxide anions, hydroxyl radicals and lipid hydroperoxides [187,189]. Collectively, all these study strongly suggest that lipofuscin participates to the light-induced oxidative stress.

There are considerable evidences linking lipofuscin with AMD: 1- Pigment granules are observed in early, small drusen, [134], 2- The highest density of lipofuscin is located in the perimacular area where there is the highest density of rods and where there is a loss of rods as a function of age and in AMD [71,190], 3- High levels of lipofuscin accumulation precede RPE cell death and GA [191,192], 4- The action spectra for photochemical damage to the RPE coincides with the aerobic photoreactivity of lipofuscin [188], 5- A2E-epoxides damage RPE, especially its DNA [185,186] and 6- A2E, has been shown to upregulate the expression of VEGF after blue light exposure [193].

Different in vitro studies on cell cultures confirm the photoinducible damage of retinal cells by lipofuscin. Blue light-exposed human RPE cells cultured with lipofuscin granules show early changes in their function, including decreased activity of lysosomal enzymes, a reduction in antioxidant status (decrease in activity of catalase, SOD and cathepsin (CatD), reduction if GSH levels), changes in gene expression and nuclear damage [194]. It is to note that CatD is very important for opsin proteolysis. Transgenic mouse that express an inactive form of catD exhibit bloated RPE cells with debris, basal laminar and linear deposits, followed by RPE atrophy and progressive photoreceptor cell death, as observed in AMD [195]. RPE cell treatment with AGEs induces downregulation of CatD expression and increases in AGEs are found in AMD [49], supporting the hypothesis of the roles of CatD and AGEs in the development of AMD. Cell membrane blistering, vacuolation, an exposure time-dependent loss of lysosomal stability, an increase in malondialdehyde, HNE, lipid peroxidation endproducts and oxidized membrane proteins, and loss of RPE cell viability were also observed after blue light exposure in human RPE cells cultured in the presence of lipofuscin granules [15,196].
Although A2E is only a minor component of lipofuscin, it has been suggested that it may be involved in the lipofuscin-mediated photooxidative retinal damage in AMD. The damaging effects of A2E can be explained by two properties of the compound: 1- its detergent-like structure and 2- its photoreactivity. A2E presents two hydrophobic side-arms and a positively charged polar head which confer to A2E an amphiphilic structure [197].

Human RPE cells cultured in the presence of A2E (A2E-laden RPE cells) and exposed to blue light underwent a loss of cell viability in comparison to A2E-laden cells maintained in the dark [198]. It has been demonstrated that the blue light-induced damage to A2E-laden RPE cell involved oxidative mechanisms, possibly through the formation of A2E epoxides [199,200]. Indeed, upon blue light irradiation, A2E self generates singlet oxygen, which reacts with A2E along the carbon-carbon double bonds of the hydrophobic side arms to form A2E epoxide [199]. A2E disrupts RPE cell membrane integrity [198]. The mechanism by which A2E penetrates the membranes is certainly influenced by electrostatic interactions between the positively charged hydrophilic head of A2E and the negatively charged phosphatidylserine of the membranes. It is unlikely the singlet oxygen which alters cells since singlet oxygen diffuses only a short distance in the cells. Moreover, singlet oxygen generated by A2E is quenched by A2E itself to generate A2E-epoxide. A2E induces loss of membrane integrity, altering membrane permeability [201] through solubilization of the lipid bilayer [202]. Nor are lipids the only cellular macromolecules altered by A2E-epoxides. DNA is one of the target of the photooxidative effects elicited in A2E-laden RPE by blue light illumination: A2E epoxide induces DNA lesions through oxidation of guanine bases [203]. Proteins are also modified by A2E since the electrophile part of A2E-epoxides form conjugates with the sulphydryl and amino groups of proteins. A2E localize predominantly to the RPE lysosomes, inducing an increase in lysosomal pH [198]. It inhibits lysosomal degradative functions in RPE cells, especially that of proteins and glycosaminoglycans [204]. A2E also compromises RPE cell function by inhibiting lysosomal degradation of photoreceptor phospholipids [204,205]. A2E induces RPE cell apoptosis by specifically targeting the mitochondrial enzyme cytochrome oxidase C [206] and inhibiting mitochondrial respiration [198]. A2E detaches proapoptotic proteins from mitochondria and induce apoptosis in mouse RPE cell cultures [206], confirming that lipofuscin/A2E-mediated RPE cell death involves an apoptotic process. Moreover, Sparrow et Coll. suggested that A2E-induced RPE cell apoptosis is executed by a proteolytic caspase cascade involving caspase 3 and provided evidences that blue light-exposed A2E-laden RPE cells may be protected RPE by Bcl-2 [207,208]. But, A2E is 2 orders of magnitude less photoreactive than lipofuscin and is relatively not abundant in lipofuscin granules, making the contribution of A2E to lipofuscin toxicity controversial in
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RPE. In addition, it has been shown that RPE dysfunction caused by A2E is likely to originate prior to A2E incorporation into the lipofuscin granules. Altogether, these studies suggest that other components contained in the lipofuscin granules may be responsible for the lipofuscin photoinducible retinal damage.

Melanin granules in RPE cells and choroidal melanocytes absorb light, preventing excessive light scattering. Melanin has been also been proposed to participate to light-induced oxidative stress in AMD. But the action spectrum for blue light damage does not coincide with the absorption spectrum of melanin nor its action spectrum for the uptake of oxygen. In addition, the spatial distribution of melanin does not coincide with the area damage within the retina in AMD and the content of melanin in the retina decreases with age [209]. There is an aged-related increase in the rate of photo-dependent oxygen uptake and the induced O2 uptake is six times faster for lipofuscin than for melanin [188]. Altogether, these studies strongly suggest that melanin is unlikely to mediate photooxidative damage in AMD.

Macular pigments have been shown to be protective [210]. Among the macular pigments, lutein and zeaxanthin are components that quench the reactive singlet oxygen and form an optical filter to cut off the most damaging blue light before reaching the sensory neurons [211,212]. Macular pigments have a broad band absorbance spectra peaking at 460 nm. Therefore, they may be effective at reducing the potentially damaging effects of lipofuscin whose photoreactivity peaks at 450 nm in elderly adults [155]. It has been estimated that they may absorb about 70% of incident light at this wavelength. Their topographical distribution (particularly dense at the fovea) may explain the pattern of cell loss in early AMD [213].

The photopigments themselves have been proposed to play a role in photochemical damage of the retina, especially of the photoreceptors. The action spectrum for photoreceptor damage was similar to that of rhodopsin [214]. In addition, the degree of light-induced damage of photoreceptors positively correlates with pre-exposure of rhodopsin to light. Moreover blue light is 50-80 times more efficient at inducing photoreceptor damage than green light. Blue light promotes the photoisomerisation of all-trans-retinal, which leads to the regeneration of rhodopsin and an increase in phototransduction, leading to photoreceptor apoptosis [215].

3c. Roles of the fatty acids

Lipids are preferential targets of oxidative stress in retinas so that, cell membranes are prime targets for ROS-induced damage. Membranes contain PUFAs, whose double bonds are a source of electrons, which in turn, produce lipid peroxyl radicals that perpetuate the electron appropriation. PUFAs account for about 50% of the lipid bilayer of rod outer segment membranes
and proteins make up the remaining 50% in photoreceptors. Docosahexanoic acid (DHA) (22:ω3) which makes up approximately 50% of the rod phospholipids, is highly susceptible to lipid peroxidation. The membrane lipids of RPE contain about 25% of 22:ω3. The lipid peroxidation is region- and age-dependent: the susceptibility of the posterior pole of the retina was positively correlated to age, whereas that of the peripheral part of the retina was not [216]. In addition, lower levels of DHA have been reported in the macula region compared with the peripheral retina, suggesting that the macula is faced with a greater oxidant challenge than the peripheral retina [217].

It has been shown that high intake of PUFAs is associated with a higher risk of late AMD, but not with early AMD [104,218,219]. In particular, high linolenic acid intake is associated with almost 50% increased risk of AMD, whereas high DHA intake is associated with a 30% increased risk of AMD. High intake of ω-3 fatty acid and fish were inversely associated with risk for AMD, when linolenic acid intake is low [220].

V. Roles of enzymatic and non-enzymatic antioxidants in the defense and prevention of RPE cell degeneration and AMD

Age-related decreases in the function of anti-oxidant systems have been proposed to play a major role in RPE degeneration during normal aging and AMD.

1. Antioxidant enzymes

SODs, catalase and glutathione-Px are antioxidant enzymes that are present in both photoreceptors and RPE. There is age-related decline (from the first to the eight decade) in the activity of SOD in the macula [221]. SOD activity is similar in RPE in eyes with and without AMD [162] and no association can be detected between systemic SOD activity and AMD [222], suggesting that SOD does not play an important role in the protection against AMD. This greatly contrasts with the phenotype of SOD2-deficient mice. Morphometric analysis shows that the photoreceptor layer is thinner centrally in the early states (10 days) and then all retinal layers apart from the RPE were thinner in the mutant animals. In older animals, mitochondrial morphologic abnormalities are found predominantly in RPE.

Data on the catalase activity and AMD are largely inconsistent. Frank et Coll., demonstrated that catalase decreases in RPE cell cytoplasm and lysosomes with age and AMD [223]. In contrast, the group of Hjelmeland showed no significant decline of catalase at the mRNA and protein levels with age [224]. Moreover, a decrease in the enzymatic activity of catalase with age
and age-related disease of the macular RPE has been observed by Liles et Coll., [162], whereas De La Paz et Coll., showed that there is an age-related decline in the activity of catalase in RPE, but no age-related decline in its activity of the central or peripheral neural retina [221]. Altogether these studies suggest that catalase is unlikely to play a major role in the antioxidant defense system of the retina in AMD.

In normal retina, there is no effect of age on the activity of glutathione-Px [221]. But, higher levels of plasma glutathione-Px are associated with a nine-fold increase in the prevalence of late AMD, and are unassociated with early AMD [222]. This indicates that glutathione-Px is one of the strongest indicators of AMD identified to date. Reduced levels of plasma glutathione reductase is associated with AMD, suggesting a role of GSH regeneration in AMD [225].

2. Vitamin C, E and A

Ascorbate is the most effective aqueous-phase anti-oxidant in human blood. Vitamin C has been shown to suppress A2E-epoxidation in RPE cell cultures with a corresponding reduction in the incidence of DNA damage and cell death [203]. In addition, vitamin C supplements which result in raised retinal ascorbate levels, were protective against photoreceptor light damage (only if given prior to light exposure) in mice [226], suggesting that it may protect RPE degeneration in AMD. However, no significant protective effect for dietary ascorbate alone was found in AMD patients [227].

Vitamin E is the major antioxidant of cellular membranes. Photoreceptor outer segments and RPE contain high amounts of one of the four forms of vitamin E, α-tocopherol, RPE content 4 to 7 times higher than of the neural retina. In addition RPE content of vitamin E increases with age, possibly as a response to increasing oxidative stress [228]. There are many in vitro and animals studies supporting the hypothesis of a major role of vitamin E in the antioxidant defense of RPE cells. Vitamin E produces a more pronounced decrease in A2E-epoxidation than vitamin C, in RPE cell cultures [186] and can limit oxygen-induced oxidative damage of bovine rod outer segment [229]. In addition, vitamin E deficiency results in retinal degeneration in primates [230] and an increased vulnerability to retinal light damage [231]. However, no significant inverse association has been found between dietary intake of vitamin E and AMD [219].

Vitamin A (retinol) is essential for vision, because it is the precursor of 11-cis-retinal necessary for the regeneration of rhodopsin. Three oxidative states exist for vitamin A: retinol, retinal and retinoic acid. No significant association between total dietary vitamin A (pro-vitamin A carotenoids plus retinol) or simply dietary retinol, and early AMD has been found so far [232].
Carotenoids are naturally occurring pigments that have the ability of capture radiant energy. They are antioxidant because of their ability to quench singlet oxygen, interact with free radicals and prevent lipid peroxidation. Lutein and zeaxanthin, named macular pigments, are present within the photoreceptors and the interneurons of the inner plexiform layer of the retina. Direct oxidation of lutein and zeaxanthin have been reported in human retina, suggesting that they do act as antioxidant [233]. In addition, it has been calculated that macular carotenoids can reduce by 40% the amount of blue light incident on the photoreceptors of the fovea [234]. Different studies showed that high dietary intake of carotenoids, especially intake of $\alpha$-carotene, $\beta$-carotene and provitamin A carotenoids, protects against AMD or reduced risk of large drusen at 5 years follow-up [219,232]. It has been reported that individual who accumulates large quantities of lutein and zeaxanthin in the crystalline lens and the retina is less likely to develop cataract and AMD. This is supported by observations that cataract is associated with increasing risk of AMD [100].

3. Glutathione

GSH is a tripeptide, $\gamma$-Glu-Cys-Gly which serves as both a major substrate for the enzymatic antioxidant defense system, including glutathione peroxidase, and as a direct antioxidant. The stabilized thiol group of GSH is oxidized to form a disulfide bond between two molecules of GSH (GSSG) and thus serves as an antioxidant. The plasma concentration of GSH decreases with age. In parallel, the GSH decrease, oxidized GSH, GSSG, increase as a function of age, indicating that there is a loss of GSH and oxidation of the circulating thiol-disulfide pool of GSH during aging. In addition, there is a trend toward lower plasma GSH in the AMD patients [163] and AMD is associated with significant reduced levels of plasma GSH reductase [225]. In addition, higher levels of plasma glutathione-Px are associated with a nine-fold increase in the prevalence of late AMD, and are unassociated with early AMD [222]. Therefore, it has been suggested that GSH may play a major role in RPE degeneration and proposed that induction of GSH synthesis may be of particular interest in therapeutical strategies against RPE cell degeneration in AMD.

In vitro studies, using preincubation of RPE cells with a mixture of amino acid precursors for GSH synthesis, glutamate, glycine and cysteine, show protection of cells from subsequent t-BHP induced cell apoptosis [163]. Alone, cysteine or glutamate and glycine without cysteine, have no protective effects against oxidative stress-induced RPE cell death [173].

Protection against oxidant focuses largely on the classical enzymatic antioxidant defense system, with ROS inactivating enzymes, such as catalase, superoxide dismutase and glutathione-Px. As mentioned above, not all these enzymes are involved in protection of RPE against oxidative stress and/or
associated with decreased risk of AMD. Recently, an alternative and possibly more effective strategy for combating the toxicity of ROS has focused attention: the induction of a family of phase 2 detoxification enzymes [235]. These proteins include glutathione S-transferases, thioredoxin, thioredoxin reductase 1, NADPH quinone reductase 1, UDP-glucuronosyltransferases, heme oxygenase 1, oxidoreductase (NQO1), \(\gamma\)-glutamylcysteine synthetase, epoxide hydrolase and glutamate cysteine ligase, which is the rate limiting enzyme for GSH synthesis and ferritin. These enzymes that display chemically antioxidant properties, share common regulatory mechanisms and are highly inducible by different agents, including dietary components. Transcription of the phase 2 genes involves a common promotor element, the antioxidant response element (ARE). Therefore cell treatment with an inducer of the Phase 2 enzymes might be an indirect way to combat lethal oxidative stress. It was shown that preincubation with the phase 2 detoxification inducer, dimethylfumarate (DMF), provides a protection against peroxide- and t-BHP-induced RPE cell death [163,172,236]. The group of Talalay recently showed that treatment of RPE cells with sulforaphane, the most potent naturally occurring phase 2-inducing activity, results in an increase of GSH trough the activation of \(\gamma\)-glutamylcysteine synthetase, and in the protection against t-BHP-induced lethal oxidative stress [237]. This indirect antioxidant also protects RPE cells from lethal effects of HNE and peroxynitrite [237]. More recently, it has been demonstrated that Sulforaphane also protects RPE cells from light-induced oxidative damage (UVA light and fluorescent light) [238]. Moreover, the degree of cell protection is also correlated with elevated NADPH quinone oxidoreductase. Elevation of GSH levels was shown to be important for the protective effects of Sulforaphane against phototoxicity in RPE cells as it is in t-BHP-exposed RPE cells [237,238].

In conclusion, all these studies strongly suggest that GSH and/or inducers of GSH synthesis may be of clinical interest in the protection of RPE against oxidative stress-induced degeneration during normal aging and AMD.

4. Association of direct antioxidants with Zinc

Zinc (Zn), which plays an important role in the antioxidant defenses, is the most abundant trace element in the human eye. Indeed, Zn does not act directly as an antioxidant, but as a cofactor for CuZn-SOD because it is involved in the regulation of catalase activity [239]. In addition, Zn induces the synthesis of metallothionin, a scavenger of hydroxyl radicals. It is to note that lower concentration of metallothionin is observed in the RPE macular region than in the RPE peripheral part and that there is an age-related reduction in the macular RPE content of metallothionin [240].

The Age-Related Eye Disease Study (AREDS) recently found that AMD patients with advanced cases of atrophic AMD or vision loss due to exsudative
AMD in one eye, who take daily supplements containing high doses of vitamin C, vitamin E, \( \beta \)-carotene and Zn can lower the risk of developing the more severe form of AMD over a five-year period [105].

The effects of high doses of Zn even in a combination of antioxidant vitamins are quite intriguing since Zn has been recently shown to be highly toxic for RPE cells [241]. Zn induces both necrosis and apoptosis which were completely inhibited by the antioxidant, N-acetylcystein (NAC), confirming that Zn mediates its lethal effect through ROS generation.

It is to note several important facts: 1- Two previous studies showed that there is no protective effect of dietary of \( \beta \)-carotene alone [227,242] and it is not found in the human retina, questioning the exact role of \( \beta \)-carotene in association with antioxidant in the protection against AMD, 2- Intake of high amount of \( \beta \)-carotene similar to that is used in the AREDS study (15 mg/day), may increase the risk of developing lung cancer in cigarette smokers, 3- the daily dose of 400 IU of vitamin E, which is used in the AREDS study, may result in a faster progression of vision loss for patients with common forms of retinitis pigmentosa.

5. Neuroprotective factors

5a. Growth factors

It has been known for many years that growth factors are expressed in, and for some of them, secreted by retinal cells [243,244]. Although, growth factors are, by definition, factors that induce (or sometimes inhibit) the growth of cells, different studies have showed that growth factors may play important roles in retinal diseases, such as retinal degeneration, through a neuroprotective effect. First, the group of LaVail showed that photoreceptor degeneration in inherited dystrophy may be delayed by injection of FGF1 or FGF2, in the animal model of RCS rat [245]. The survival-promoting neurotrophic activity of FGF1 and FGF2, and other factors, including brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF), was then demonstrated in a constant light-induced photoreceptor cell death [246-248] and ischemic injury of retina [249] in rats. The demonstration that apoptosis was the dominant cell death process of photoreceptor degeneration in the RCS rat [250] and in three different mouse models [140], suggested that exogenous neurotrophic factors, such as FGF1 and FGF2, may be of therapeutic potential in human retinal diseases in which photoreceptor degenerate. The idea to use exogenous FGFs to rescue photoreceptors from degeneration was rapidly rule out, since FGFs are also potent angiogenic factors and therefore may induce CNV. This was confirmed by showing that exogenous FGF2 induces the proliferation of vascular endothelial cells of the choroid [251]. But, the idea of subtle manipulation of endogenous FGFs, FGFs-induced antiapoptotic
signaling, or antiapoptotic transduction common to FGFs, BDNF, and/or CNTF, if it exists, remains quite attractive, since it has been demonstrated that inhibition of endogenous FGFs participate to photoreceptor degeneration and apoptosis in the rat model of light-induced retinal degeneration [252].

FGF2 has been shown to rescue RPE cell apoptosis due to serum starvation, a process associated with oxidative stress by the generation of superoxide [253]. FGF2-mediated protection of RPE cells was dependent on Bcl-x1 synthesis in these cell culture conditions [254]. This suggests indirectly that FGF2 may protect RPE cells from oxidative damage. There is also increasing evidences that growth factors can protect RPE cells from direct oxidative damage. HGF pretreatment inhibits ceramide/H$_2$O$_2$-induced apoptosis and reduces the accumulation of ROS, the activation of caspase-3, and the increase in MPT, and prevented the reduction in catalase activity and expression in RPE cells [175]. Lens epithelium-derive growth factor (LEDGF) is present in RPE cells, predominantly in the nucleus. LEDF protects RPE cells against H$_2$O$_2$ exposure and UVB irradiation, and protects the DNA from single-strand breakage induced by these two inducers of oxidative stress [255].

Sub-RPE deposits have been implicated as either a cause or a consequence of AMD. Therefore, it remains possible that prevention or modification of these deposits may provide a novel therapeutic approach of AMD. Amin et coll., recently showed that TNF-α reduces deposit formation of RPE in cell cultures [256]. It is unlikely that the mechanism of action of TNF-α may be due to a direct antioxidative process since it has been shown that low doses of TNF-α or H$_2$O$_2$ alone do not induced RPE cell death, while a combination of low doses of TNF-α and H$_2$O$_2$ induced RPE cell death [257]. Because there is increasing evidence that matrix metalloproteinases (MMP) and their tissue inhibitors (TIMPs) may play important roles in the pathogenesis in AMD [258], the effects of MMP 2 were tested. MMP2 does similar effects than TNF-α [256]. It is possible that the mechanism of action of TNF-α may be due to the stimulation of MMP expression. Thus, it is hypothesized that subtle modulation of MMP activity would provide an opportunity to promote deposit clearance and prevent AMD progression.

Oxidative stress also alters the expression of growth factors in RPE cells. Peroxide changes the expression pattern of PEDF and VEGF [54]. Oxidative stress induces a disequilibrium between anti-angiogenic and angiogenic factors, and increases the angiogenic potential of vascular endothelial of the choroid and decreases RPE cell survival.

5b. Docosahexanoic acid

Docosahexanoic acid (DHA) (22:ω3) is a lipid peroxidation target in oxidative injury. Diet-supplied DHA is initially taken by the liver and then distributed to the organs through blood lipoproteins. Photoreceptor outer
segment content the highest concentration of DHA of any cell type [259]. It represents approximately 50% of the rod phospholipids and about 25% of those of RPE. Lower levels of DHA have been reported in the macula region compared with the peripheral retina, suggesting that the macula is faced with a greater oxidant challenge than the peripheral retina [217]. The lipid peroxidation is region- and age-dependent, with the susceptibility of the posterior pole of the retina positively correlated to age, whereas that of the peripheral part of the retina is not [216]. DHA is strongly present in fish oil and different studies showed that high intake of ω3 fatty acids and particularly DHA was associated with reduced risk of AMD [104] and high dietary consumption of fish and/or ω3 fatty acids is inversely associated with risk for AMD [103]. Altogether, these data strongly suggest that ω3 fatty acids and particularly DHA may play a pivotal role in the antioxidant defense of the retina.

However, DHA mediates neuroprotection in photoreceptors [260,261], whereas oxidative stress generates neuroprostane from DHA in the brain [262]. Recently, it has been isolated and characterized a 10,17S docosatriene from DHA from RPE cells, that possesses neuroprotective effects in oxidative stress-exposed RPE cells through the inactivation of the oxidative stress-mediated proapoptotic signaling [257]. This neuroprotective mediator of DHA is called neuroprotectin D1 (NPD1). NDP1 counteracts H$_2$O$_2$/TNF-α oxidative stress-triggered apoptotic RPE DNA damage. It also upregulates the antiapoptotic proteins Bcl-xl and Bcl-2 and decreased proapoptotic proteins Bax and Bad expression. The antiapoptotic effects of NDP1 seems to be mediated through the inhibition of caspase-3 activation [257].

5c. Other molecules

Recently, prostaglandines have been shown to protect RPE cell from oxidative stress. RPE cell apoptosis induced by either hydrogen peroxide or t-BHP is prevented by a pretreatment of 15-deoxy-delta 1é, 14-Prostaglandine J2 (15d-PGJ2) [263]. The antiapoptotic effect of 15d-PGJ2 is mediated by a decrease in the generation ROS and a significant restoration of the mitochondrial membrane potential.

5d. Is there common antiapoptotic signaling pathways for RPE cells and photoreceptors exposed to oxidative stress?

Caspase-3 activation was observed and/or involved in RPE cell apoptosis induced by different stress including oxidative stress such as t-BHP [172], C2 ceramide [175], blue light [208], and metabolic stress such as serum starvation [254]. Moreover, the two antiapoptotic proteins Bcl-2 and Bcl-x have been shown to inhibit the release of cytochrome c, which is associated with the activation of caspase-3 from mitochondria and Bcl-xl has been shown to be cleaved by caspase-3 [264,265]. Bcl-2 gene is not normally expressed in the
retina. In transgenic mice expressing Bcl-2 gene there is a preservation of the photoreceptors [266] after light damage and in degenerating rd mice, suggesting a role of antiapoptotic members of the Bcl-2 in the survival of degenerating retina. In addition, the role of the antiapoptotic proteins of the Bcl-2 family, in the protection of RPE cells, has been often described. For example, a protective effect of Bcl-2 in blue light- and H$_2$O$_2$/TNF-α-induced RPE cell apoptosis [208,257] and of Bcl-xl in H$_2$O$_2$/TNF-α- and serum starvation-induced RPE cells [254,257] has been observed. Therefore, one may hypothesize that Bcl-2/Bcl-xl may be of central importance in the protection of both RPE cells and photoreceptors exposed to oxidative stress. This hypothesis has been recently confirmed in RPE cells, where Bcl-2 overexpression increases cell survival after exposure to H$_2$O$_2$ [267]. Thus, modulating proteins of the Bcl-2 family might be likely contributing to potential new therapeutic strategies against RPE and photoreceptor degeneration.

Because exogenous FGF2 and endogenous FGF1 have been demonstrated to induce the expression of Bcl-2 and Bcl-xl in photoreceptors and RPE cells, respectively [253,268-270] a major and common survival pathway to retinal cells exposed to different oxidative injuries, may be under the control of FGFs-regulated Bcl-2/Bcl-xl expression. This hypothesis is supported by data showing that an endogenous FGF1 neuroprotective effect is linked to Bcl-2 expression [271]. Moreover, oxidative stress-induced RPE cell apoptosis is accompanied by a decrease in the expression of the antiapoptotic protein, Bcl-2 and an increase in the expression of p21 and p53 [166], while the antiapoptotic effects of FGF2 on serum-depleted cortex neurons has been shown to be mediated through an attenuation of the changes in the expression of Bcl-2 and p53, and caspase-3 activity [272]. Altogether, these data strongly suggest that the antiapoptotic effect of FGF2 may be mediated by such processes in photoreceptors and/or RPE cells exposed to oxidative stress. The exact protective molecular mechanisms of the growth factors, such as BDNF, CNTF, LEDGF, or HGF on retinal cells exposed to oxidative stress also remains to be defined. But, the common antiapoptotic effects of all these RPE protective molecules described above, including growth factors and fatty acids (DHA or NDP1), suggest a common survival signaling.

It is clear that alterations in gene expression and enzyme activity induced by cellular stress such as oxidative stress are mediated through the interplay of multiple signaling pathways. ROS cause damage to macromolecules, but they also serve as signaling molecules to stimulate protein kinase cascades coupled not only to cell death signaling, but also to adaptive response to oxidative stress, including, antioxidant responses, control of the cell cycle, or inflammatory gene expression. Mitogen-activated protein kinases (MAPKs) cascade is one of the most ubiquitous signal transduction systems and is rapidly activated in response to a variety of cellular stimuli, including cellular
stress and cell death. The antiapoptotic proteins of the Bcl-2 family have been shown to be under the control of two of these MAPKs, the extracellular-signal-regulated kinases 1 and 2 (ERK1/2), in RPE cells exposed to different stress. Therefore, it is tempting to hypothesize that ERK1/2 signaling may play a central role in transmitting and integrating signaling from different antiapoptotic molecules for inducing survival in RPE cells or photoreceptors, exposed to various injury, including oxidative stress. ERK1/2 mRNA is present in all the nuclear cell layer of the retina and in the RPE [273]. Recent data on the apoptotic signaling transduction of retinal cells exposed to different oxidative stresses show the activation of ERK1/2 [274,275]. In these studies, inhibition of ERK1/2 activation has been shown to sensitize cells, suggesting that ERK1/2 participate to the antiapoptotic signaling pathway induced by oxidative stress. But, there is also growing evidences implicating these kinases in the promotion of apoptosis by different cell death inducers, including oxidants such as H$_2$O$_2$ and glutathione depletion [276,277]. Difference in outcome resulting from the activation of ERK1/2 may depend not only upon the environmental context of the cell, or the cell type expressing ERK1/2, but also upon the nature, the severity and the kinetics of the oxidative injury. Issues such as this remain to be explored in photoreceptors and RPE cells.

In addition, it has been recently shown that overactivation of two other members of the MAPK family, JNK-1 (c-jun NH$_2$-terminal kinase 1) and P38 kinase occurs in parallel to serum-depletion induced cell death in RPE cells [278]. Overactivation of JNK-1 and P38 kinase was also detected during pharmaceutically-induced RPE cell death [279], suggesting that the signaling pathways of these two kinases are common to RPE cell death-signaling pathway induced by various stress stimuli implicated in RPE cell death. This is confirmed by the recent data showing an activation of the P38 kinase in RPE cells exposed to a lethal dose of the oxidant agent, hydroquinone [280]. JNK-1 and P38 kinases are the major transduction molecules that play a pivotal role in both the oxidative stress-induced bipartite response, which can lead to either neurodegeneration (apoptosis) or neuroprotection (defensive-protective adaptations), depending on the cellular and environmental conditions as well as cooperation with other signaling pathways. Interestingly, AMD shares numerous pathological characteristics, including inflammatory responses and oxidative stress (lipid peroxidation, amyloid β deposits production of AGE antioxidant responses), with other neurological diseases associated with aging, such as Alzheimer’s disease (AD). Overactivation of JNK-1 and P38 kinase was detected in AD. These two MAPKs are implicated in both early and late AD stages by controlling gene induction of several antioxidant enzymes, and the phosphorylation of tau protein and the formation of neurofibrillary tangles, respectively [281]. Therefore, it will be of value to study the activation and the roles of these MAPKs in photoreceptor- and RPE cell degeneration during AMD.
VI. References


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