Mechanisms of protein aggregation in the retinal pigment epithelial cells

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1. ABSTRACT

The pathogenesis of age-related macular degeneration (AMD) essentially involves chronic oxidative stress, increased accumulation of lipofuscin in retinal pigment epithelial (RPE) cells and extracellular drusen formation, as well as the presence of chronic inflammation. The capacity to prevent the accumulation of cellular cytotoxic protein aggregates is decreased in senescent cells which may evoke lipofuscin accumulation into lysosomes in postmitotic RPE cells. This presence of lipofuscin decreases lysosomal enzyme activity and impairs autophagic clearance of damaged proteins whichshould be removed from cells. Proteasomes are another crucial proteolytic machine which degrade especially cellular proteins damaged by oxidative stress. This review examines the cross-talk between lysosomes, autophagy and proteasomes in RPE cell protein aggregation, their role as a possible therapeutic target and their involvement in the pathogenesis of AMD.

2. INTRODUCTION

AMD is a complex disorder of the eye, representing the leading cause of blindness in the developed countries. It has a multifactorial etiology and results in a progressive loss of central vision of the eye in the elderly and thus it is becoming a major public health issue (1-3). The disease affects the macula, which is located in the central area of the retina. AMD is characterized by degeneration of the macular retinal pigment epithelial (RPE) cells, Bruch's membrane, and choriocapillaris (4). One of the main functions of RPE cells is to take care of neural cells, rod and cones. In senescent RPE cells this ability is weakened causing secondary adverse effects on neural retina and ultimately leading to loss of vision. AMD results in central visual defects such as reduced visual acuity and contrast sensitivity and these are so severe that many patients have difficulties in coping with routine daily tasks. AMD aetiology is known to have a strong genetic component and to be associated with several environmental
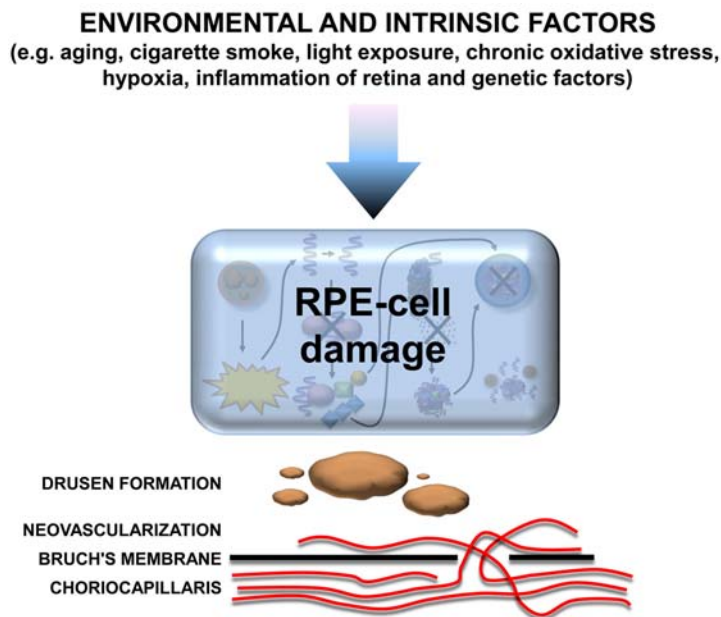
ENVIRONMENTAL AND INTRINSIC FACTORS
(e.g. aging, cigarette smoke, light exposure, chronic oxidative stress, hypoxia, inflammation of retina and genetic factors)

RPE-cell damage

DRUSEN FORMATION

NEOVASCULARIZATION

BRUCH'S MEMBRANE

CHORIOCAPILLARIS

Figure 1. AMD is a multifactorial disease that is characterized by degeneration of the macular retinal pigment epithelial (RPE) cells, Bruch's membrane, and choriocapillaris. AMD aetiology is known to be associated with many environmental risk factors and genetic components AMD. It is divided into early and late diseases, as well as the atrophic and exudative degeneration categories. The exudative AMD comprises of choroidal neovascularization that develops in 15-20% of AMD cases. A high amount of drusens predict progression and severity of AMD.

risk factors such as aging, smoking, obesity, hypertension and hypercholesterolemia (Figure 1; 5-7).

AMD is divided into early and late diseases, another subdivision is into atrophic and exudative degeneration categories (8). The exudative AMD consists of choroidal neovascularization that develops in 15-20% of AMD cases. New vessels tend to sprout from the choroidal capillaries through Bruch's membrane into the sub-RPE space or into retinal layers and these cause a rapid and permanent loss of visual acuity if not treated. Anti-VEGF (vascular endothelial growth factor) intravitreal injections were observed to prevent effectively blindness in exudative AMD (9). However, most of the AMD patients (80%) suffering from atrophic AMD are still without any effective treatment. The RPE cells live under conditions of chronic oxidative stress and this evokes detrimental cellular effects e.g. protein aggregation during the aging process. A hallmark of AMD is the accumulation of lysosomal lipofuscin in RPE cells and the presence of extracellular drusen deposits between the basal lamina of the RPE and the inner collagenous layer of Bruch's membrane. A high level of lipofuscin and drusens predict the progression and severity of AMD (Figure 2; 10-11). In this review, we will discuss role of protein aggregation in the pathogenesis of AMD.

3. RETINAL PIGMENT EPITHELIUM (RPE)

The postmitotic RPE cells constitute a polygonal monolayer between the neurosensory retina and the fenestrated capillaries of the choroid. RPE has multiple functions (I) absorption of light energy, (II) transport of metabolites and nutrients between photoreceptors and choriocapillaris, (III) expression of growth factors for photoreceptors, (IV) regulation of homeostasis of the ionic environment, (V) phagocytization of the shed tips of photoreceptor outer segments, (VI) regulation of visual cycle and (VII) the creation of the blood–retinal barrier (12). At the same time while performing these vital functions, the RPE cells are exposed to chronic oxidative stress that leads to cellular dysfunction and degeneration during the aging process (13-15). Degeneration of the RPE cells is one of the most important hallmarks of AMD (16). Clinically, it can be observed by pigment mottling, accumulation of intracellular lysosomal lipofuscin and extracellular drusen deposits (Figure 2; 16-17). In senescent cells, especially in postmitotic cells such as the RPE cells, increased oxidative damage evokes protein misfolding and aggregation (18). One characteristic indication of the cellular aggregation is the progressive lipofuscin accumulation in cell lysosomes of RPE as a terminal pathway of phagocytosis of shed photoreceptor outer segment disks (19).

4. LYSOSOMAL LIPOFUSCIN

Lipofuscin is a highly crosslinked aggregate consisting of oxidized proteins and lipids. Due to these properties, it is believed that, once formed, lipofuscin is not degraded by proteasomal or lysosomal enzymes (see later) or transported into the extracellular space via exocytosis (20-22). However, excess lipofuscin accumulation may secondarily disturb cytoplasmic protein degradation

Figure 2. Fundus autofluorescence image from degenerated macula indicating increased lipofuscin accumulation with increased autofluorescence signal (A) and colour photograph from degenerated macula in AMD patient with abundant yellow/white drusens (B).

processes and lead to increased transcytosis and exocytosis in RPE cells (23-25). Many previous studies have shown that lipofuscin compounds such as A2-E (N-retinylidene-N-retinylethanol-amine) fluorophore and its precursors increase oxidative stress and have toxic properties in RPE cells (26-30). These harmful compounds are formed by the mixture of oxygen-containing moieties within photooxidized A2-E reactions during the visual cycle which react to form peroxidation products of proteins and lipids (31). Constant oxidative stress, decreased antioxidant capacity and molecular chaperone function and disturbed protein degradation are thought to be major factors that ultimately lead to RPE senescence and death (Figure 3; 18).

It is well known that old cells have a 10-fold iron concentration compared with young cells (32). Increased iron accumulation is also observed in the retinas of AMD patients (33-34). Once iron is released into lysosomes, it reacts with hydrogen peroxide and forms hydroxyl radicals in the so-called Fenton reaction. Subsequently, highly reactive radicals cross-link lysosomal proteins, lipids and carbohydrates that evoke lipofuscinogenesis and destabilization of lysosomal membranes (35)

Lysosomes are currently being targeted to prevent detrimental lipofuscin formation. It has been speculated that iron chelators that easily penetrate through cellular membranes could inhibit redox-active iron and lipofuscinogenesis. The literature reveals that two iron chelators, spermine or desferrioxamine, might be able to prevent lipofuscin formation and decrease oxidative stress in postmitotic cells (36). Recently, Kurtz observed that apo-ferritin, also a compound which binds to iron, could also prevent lipofuscin accumulation (37).

Small natural polyphenol molecules, such as resveratrol, quercetin and curcumin, function as hormetic compounds that evoke low level stress stimuli within cells. This has been estimated to help maintain cellular functions and increase longevity. These polyphenols are calorie restriction mimics that activate DNA repair enzymes, suppress inflammatory signaling and increase autophagic cleansing processes (38). Interestingly, oral resveratrol intervention improved visual functions and this was associated with a decreased retinal lipofuscin autofluorescense signal (39).

Antioxidants have been shown to possess efficacy in many RPE cells cultures in preventing lipofuscinogenesis, but their role in the prevention of AMD is still debatable (40-42). In summary, lysosomes are an interesting pharmaceutical target in attemps to prevent lipofuscinogenesis, but larger studies are required to prove the efficacy of antioxidants, iron chelators or polyphenols in the prevention of lipofuscinogenesis and AMD.

5. PROTEASOMAL PROTEIN DEGRADATION

The RPE cells live under chronic oxidative stress due to normal visual cycle metabolism, constant light exposure and they are also subjected to lipofuscin triggered stress. Ultimately, this creates a significant mass of cellular misfolded proteins, which are continuously being formed. Misfolded proteins have a tendency to gather into detrimental aggregates (43). In addition to stress –damaged proteins, the nonfunctional proteins may also be a result of gene mutations and disturbances in protein synthesis leading to misfolding (44-45). Prior to the ultimate aggregation cellular defense mechanism is triggered, called the molecular chaperone response (heat shock protein response), that attemps to restore proteins to their original folding state, retain their function and reduce this deleterious aggregation process (18,46). If this response is unsuccessful, misfolded proteins are tagged with a small polypeptide ubiquitin (Ub) that functions like a "stamp" and directs the complex to the ubiquitin/proteasomal protein degradation pathway (UPP; 47-48). The eukaryotic proteasome is a multicatalytic proteolytic complex present in cytosol, nucleus and microsomes, and it accounts for 1% of all cytosolic proteins (49). The proteasome recognizes and selectively degrades oxidatively damaged and ubiquitinated proteins. The most common form of the proteasome is known as the 26S proteasome, which is about 2000 kilodaltons (kDa) in molecular mass and contains one 20S core particle structure and two 19S
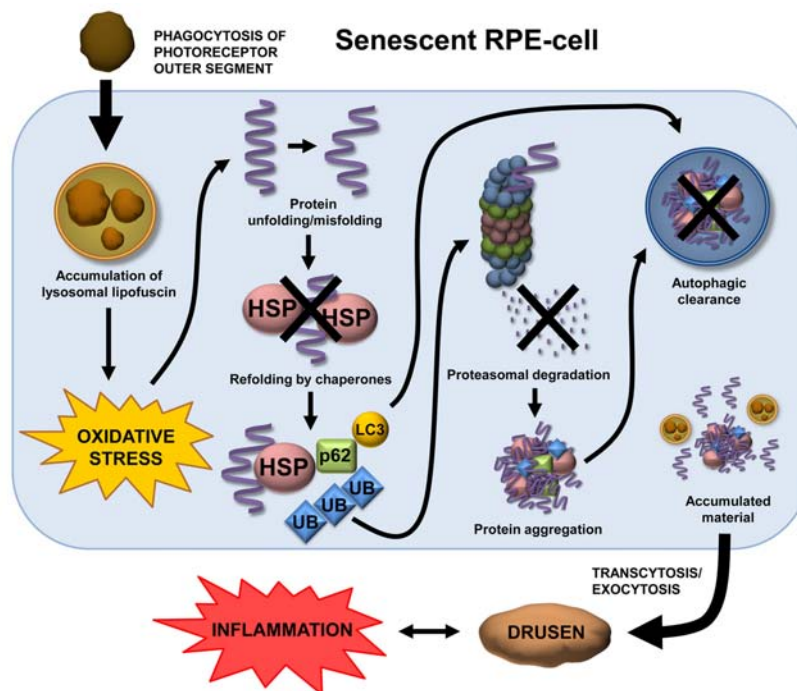
Figure 3. Schematic representation of protein aggregation in retinal pigment epithelial cells (RPE). RPE cells digest retinal outer-segment disks that are endocytosed and fused with lysosomes to be degraded. In aged RPE cells, lysosomal degradation is impaired resulting in accumulation of lipofuscin that increases oxidative stress and protein damage in the cells. Heat shock proteins (HSPs) try to repair protein damage caused by oxidative stress, but this process may be disturbed in aged cells as also are the proteasomal and autophagy protein clearance systems. Since, the efficiency of central proteolytic machines is impaired in aged cells, proteins are apparently moved via transcytosis/exocytosis to the outside of the RPE cells. This material might be involved in drusen formation.

regulatory caps (50). The 20S proteasome, a 700 kDa multisubunit enzyme complex, is a barrel-shape stack of four heptameric rings. Binding of each side of 20S particle by the 19S regulatory complex gives rise to the 26S proteasome that is responsible for the ATP/ ubiquitin-dependent degradation of oxidized proteins. Thus, proteolysis by the ubiquitin/proteasome involves two major steps: ubiquitination and degradation.

The UPP is one of the most important cellular systems to remove damaged proteins in response to oxidative stress. Ub is highly conserved from yeast to man (50). Ubiquitin was initially documented to be a regulator of protein degradation, but subsequent observations have confirmed its participation in the regulation of other cellular processes, such as endocytosis, cell cycle, signal transduction, gene regulation and DNA repair (50-52). Conjugation of Ub is a complex reaction, which is activated in an ATP-dependent manner by a ubiquitin-activating enzymes. First, E1 enzyme transfers Ub to a lysine-residue (Lys) of E2, and both E2 and the targeted substrate for destruction are bound by an E3 enzyme, inducing the transfer of Ub from E2 to a lysine amino groups of the substrate. Subsequently, E2 and E3 are released. The cyclic transfer of more Ub-molecules to the first Ub attached to the substrate is performed by these enzymes and by another enzyme – the E4 (50-51). The substrate specificity is provided by the E3 class of enzymes, which contains

thousands of Ub-ligases, each one specific for only a limited number of substrate proteins (50). Those four sequentially attached ubiquitins allow the ubiquitylated target protein to be recognized and degraded by the 26S proteasome (51). The modification of a target protein with Ub chains (polyubiquitination), attachment of a single Ub moiety (monoubiquitination) and/or oligomeric Lys63-linked Ub chains provide many different opportunities to regulate the protein degradation process in cells (51). Interestingly, recent experimental studies provided evidence for the involvement of Ub also in lysosome-dependent protein degradation system, autophagy (see later).

There is a hypothesis that the accumulation of oxidized and ubiquinated proteins is due to the decrease of proteasome activity with age (Figure 3; 50,53). In support of this hypothesis, Li *et al.*, (54) documented a decreased capacity of UPP to degrade harmful proteins in aged RPE cells. Moreover, under certain circumstances, oxidative stress itself may inactivate the function of proteasomes and up-regulate the release of proinflammatory cytokines (55) which may account for the chronic inflammation in retina and the accumulation of drusens in the space between Bruch´s membrane and RPE.

Cellular sensors, such as HSPs, detect different forms of stress and promote cellular adaptation

and survival in RPE cells (18,56). HSP expression is clearly linked to protein misfolding and proteasome inhibition (18,57). One of the most important homeostatic responses involved in maintaining longevity is the induction of heat shock proteins (HSP), an evolutionarily conserved reaction to the presence of damaged intracellular proteins (58). However, in aged cells, reduced levels of HSPs and the resultant loss of protein quality control are decreased and this poses a further challenge to proteasomal protein degradation systems. However, a common age-related feature observed in many tissues is accumulation of the Ub-conjugated proteins (59). These proteins, which have been tagged with Ub for degradation but not efficiently removed, might be detrimental to cell viability. Excessive accumulation of the Ub-protein conjugates is observed in many pathological conditions such as Alzheimer's disease, Parkinson's disease and other neurodegenerative disorders. Interestingly, increased Ub levels have been found in drusens isolated from AMD patients (60). However, the role of Ub in drusenogenesis and AMD pathogenesis still needs to be clarified.

In conclusion, decreased HSPs chaperone function and at the same time impaired proteasomal clearance favours the accumulation of detrimental protein aggregates that gather especially in postmitotic cells like RPE cells (54,57). The aggregates formed may, however, undergo autophagic clearance under certain circumstances.

6. AUTOPHAGIC CLEARANCE

Autophagocytosis, which is a specific lysosomal clearance system, was discovered by Arstila and Trump over four decades ago (61). Recently, autophagy reasearch has become one of the most excitng branches of cellular pathology. Autophagy is an intracellular catabolic process involved in protein and organelle degradation via the lysosomal pathway that has been linked to the pathogenesis of AMD (18,22,24,57). Autophagy is categorized into three different clearance systems: (1) macroautophagy, characterized by the formation of a crescent-shaped phagophore that expands to form a double membrane autophagosome, which then fuses with lysosomal vesicles and delivers the engulfed cytoplasm for degradation; (2) microautophagy, in which lysosomes invaginate and directly sequester a portion of cytoplasm; and (3) chaperone-mediated autophagy (CMA), which involves distinct cellular chaperones assisting in the uptake of specific proteins into lysosomes (62).

Microtubule-associated protein 1A/1B-light chain 3 (LC3) is a soluble protein that is distributed ubiquitously in mammalian tissues and cultured cells. During autophagy, autophagosomes can engulf cytoplasmic components, including cytosolic proteins and organelles (63). Concomitantly, a cytosolic form an of LC3 (LC3-I) is conjugated to phosphatidylethanolamine to form LC3-phosphatidylethanolamine conjugate (LC3-II), which is recruited to autophagosomal membranes. Autophagosomes fuse with lysosomes to form autolysosomes, and endo-autophagosomal components are degraded by lysosomal

hydrolases (63-64). At the same time, LC3-II in autolysosomal lumen is degraded. Thus, lysosomal turnover of the autophagosomal marker LC3-II reflects autophagic activity. Interestingly, a novel protein called p62/SQSTM1 was observed to binds directly to Atg8/LC3 to facilitate degradation of Ub protein aggregates by autophagy (65; see later).

AMD-associated stress conditions such as hypoxia, oxidative stress, unfolded protein response or inflammation are typical inducers of autophagy (15,18,57). Autophagy contributes to intracellular quality control and good cellular housekeeping, especially in the turnover of aggregate-prone proteins, a process that is extremely important in postmitotic cells, such as RPE cells. Enhanced autophagy reduces the toxicity of the protein aggregates that accumulate in many age-associated diseases, but unfortunately the autophagy activity declines during the aging process (66).

Recent observations indicate that disturbed autophagy is involved in AMD pathogenesis (24). An effective autophagic clearance system has recently been documented also in human RPE cells (24, 57). If lysosomal function becomes suppressed, as occurs during excess lipofuscin loading, then autophagic clearance is no longer functional in RPE cells in response to impaired cellular proteolysis (Figure 3; 57,67). The preservation of the autophagic activity together with functional lysosomal enzymes is a pre-requisite if one wishes to prevent detrimental intracellular accumulation of damaged proteins. (57,66). A well-functioning proteolytic machine quarantees a good capacity to handle damged protein and in this way to improve RPE cell function and retard the aging process.

7. THE P62/SEQUESTOSOME 1 AS A LINKER MOLECULE OF PROTEASOMES AND AUTOPHAGY

7.1. Multifunctional role of p62/sequestosome 1

The p62 was first characterized in polyubiquitinated protein aggregates in response to proteasomal depletion (68). Subsequently, it has been noted that p62 is expressed in many neurodegenerative and liver diseases, like Lewy bodies in Parkinson's disease, neurofibrillary tangles in Alzheimer's disease, huntingtin aggregates, Mallory bodies in hepatocellular carcinoma and alpha1 antitrypsin aggregates in liver (69-72). Interestingly, several proteins identified in the deposits in Alzheimer's disease have also been found in eye samples collected from patients with age-related macular degeneration (60). The ability of p62 to bind non-covalently to ubiquitin was first described by Vadlamudi et al., (73) but only later was it realized that p62 is one of the main molecules controlling the shuttling of ubiquitinated substrates towards proteasomal degradation (74-77).

The p62 transcription and protein levels are increased in oxygen radical stress (78). Oxidative stress activates transcription factor Nrf2 and its inactivation results in p62 depletion (79). Inhibition of proteasomal

activity also induces the synthesis of p62 (78,80). The p62, sequestosome 1 (SQSTM1) formerly named ZIP (Zeta PKC-interactin protein) or A170 murine protein (78,81) is a multifunctional multidomain protein adapter which has many roles in cell signaling, transcription regulation, receptor internalization and protein turnover. It was initially discovered as an adaptor/scaffold protein when activators and co-factors of protein kinase Cs (PKCs) were being screened. Subsequently, the p62 has been reported to play manya roles in inflammation, neurogenesis, osteoclastogenesis, adipogenesis, T-cell differentiation, and tumorigenesis (82). One of the best described functions of p62 is its involvement in signaling of atypical PKCs (aPKCs) (insensitive to Ca-ions and lipid second messengers), which leads to the selective activation of transcription factor NF-kappaB which is a major trigger for the inflammatory process (77,83-84).

In addition to being found in cytoplasm, p62 is also localized in the nucleus where it has been claimed to be involved in transcription regulation. The 400-amino-acid long p62 protein has multiple regions and domains, which allow it to have the potential for interacting with many diverse partners and ligands. Near to its amino terminus, the SH2 domain binds to Scr homology 2 (SH2). PB1 domain (Phox and Bem1p) is used in polymerization of p62. A ZZ-type domain (zinc finger) is responsible for Rip1 binding and downstream signaling. TBS (TRAF6 binding site) is a region for binding tumor necrosis factor receptor - associated factor 6 (TRAF6), followed by two PEST sequences (strongly regulating the half-life of proteins) including possible phosphorylation sites and after that LIR (LC3 interacting region) which is connected to autophagy (see later). A ubiquitin-associated domain (UBA) is located near the C-terminus of p62, enabling non-covalent binding to ubiquitin or ubiquitinated substrate proteins, leading finally to proteasomal clearance (76-77).

7.2. The p62/sequestosome 1 as a regulator of proteasomal and autophagic clearance

In normal situations, damaged, misfolded or otherwise potentially toxic proteins are degraded by the proteasome machinery. Dysfunction of ubiquitin-proteasome system might cause aggregation of damaged proteins into larger bodies called aggresomes (43). Inhibition of p62 transcription blocks proteasomal sequestration of ubiquitinated proteins and enlargement of inclusions. Therefore p62 plays a vital role in the defence of cells from the toxicity of misfolded proteins by augmenting aggregate formation (85-86). As such, the accumulation of dysfunctional proteins in bodies called aggresomes might be itself a mechanism intended to protect the cells (87). Disease progression caused by dysfunctional proteins has been shown to be accelerated in the absence of p62 due to an inability to sequester proteins in aggregates (86-87).

The ubiquitin-associated domain (UBA) enables non-covalent binding to ubiquitin or ubiquitinated substrate proteins (76). Initially, p62 proteins are polymerized with the help of PB1 domain and later TRAF6 (= TNF receptor associated factor 6) is attached to TBS in the middle of p62 along with the ubiquitin chains. These branched ubiquitin chains (K63, employing lysine, K) are then transferred from TRAF6 to substrate proteins, which finally interact with UBA-domain of p62. These complexes are then targeted to proteasomal degradation, where the N-terminal PB1 domain of p62 binds to the proteasome (Figure 3). Alternatively, ubiquitinated complexes are shuttled to lysosomes for autophagocytic degradation (Figure 3; 77,83,84, 88). It has been proposed that p62 regulates the packing and transport of ubiquitinated, misfolded and aggregated proteins, and also non-functional cell organelles for clearance via autophagy in mammalian cells (64,88).

With respect to autophagy, the most important region in p62 is LIR (LC3 interacting region), which is a general motif for docking of ATG family proteins (atg, autophagy related genes, first described in yeast), of which mammalian LC3 (microtubule-associated protein 1 light chain 3) is one subfamily (64). This points to a role of p62 in degradation of ubiquitinated cargoes and a putative role for LIR as a general motif for docking of proteins into ATG8/LC3 family proteins.

In addition, the p62 accumulation in mice genetically manipulated to be deficient for autophagy points to a link between autophagy and p62 (89). p62 interacts directly with LC3 with LRS (LC3 recognition sequence) domain. If mutations are made in p62 so that it lacks the LC3 binding site, then there is induction of inclusion body formation, similar to that seen with autophagy-deficiency (65,84,90-91). More recently, Noda et al. (92) have described a WXXL-motif responsible for the interaction with Atg8/LC3 -family proteins in p62. This motif seems to be crucial for selective autophagy.

8. TRANSCYTOSIS IN DRUSEN FORMATION

Degeneration of the RPE cells is observed by pigment mottling, accumulation of intracellular lysosomal lipofuscin and extracellular drusen deposits (16-17). Drusen formation is still a mystery, even though it has been strongly associated to the development of AMD (10). It could be argued that there is an impaired protein clearance process in the extracellular space which might results in increased secretions from the RPE cells that have lost their capacity to dispose of protein cargo within the cells themselves. These intracellular proteins may diffuse and accumulate into extracellular space and there be detected as drusens.

Transcytosis is the transport of macromolecular cargo from one side of a cell to the other within a membrane-bounded carrier(s). It is a process utilized by multicellular organisms to selectively move material between two different environments while maintaining the distinct compositions of those environments (93). Transcytosis is a rather ubiquitous transport process and it also occurs in RPE cells (94). The most extensively studied aspect of transcytosis is its presence in epithelial tissues, which form cellular barriers between two environments, such as neural retina and

choriocapillaris separated by the polarized RPE cell layer. In the polarized cell type net movement of material can be in either direction, apical to basolateral or vice versa, depending on the cargo and particular cellular context of the process (93). Apparently, cargo in the transcytotic pathways seems able to avoid degradation in RPE lysosomes (25,94). Transcytosis may be a selective or nonselective process and it is not limited to macromolecules (93).

Once molecular chaperones, lysosomes, autophagy and proteasomes have suffered an impaired capacity to maintain normal cellular protein turnover in aged RPE cells, transcytosis might shuttle excess cargo to the extracellular space and initiate the formation of drusens (Figure 3; 18). This hypothesis is supported by a recent observation that inhibition of lysosomal degradation by lipid peroxidation-related protein modifications induced apical-to-basolateral transcytosis of undegraded POS proteins in human RPE cells *in vitro* (94). Immunohistochemical analyses have revealed that the drusen is composed of many RPE originated proteins that normally regulate inflammatory and proteolytic processes (24,95). Their presence in drusens may be an indication of decreased autophagy and increased transcytosis and exocytosis in RPE cell and thus explain their place in AMD development (18,24,94).

9. COMPLEMENT SYSTEM IN DRUSEN FORMATION

In parallel with the protein accumulation originating from RPE cells, drusen formation may be regulated by the complement system. Recent genome-wide association studies on large AMD case–control cohorts have helped in mapping genes in the complement cascade that are involved in the regulation of innate immunity and AMD (6-7). Major associations have been observed in the complement system (*CFH, CFB, C2* and *C3*) and AMD in several populations. These findings clearly indicate that genetic risk factors, specifically those related to the complement system, have been linked to AMD. The complement system is an ancient component of the host immune defence. The complement system is divided into the classical, alternative and lectin binding pathways**.** C-reactive protein (CRP) is a major acute phase protein that is a key regulator of the complement system alternative pathway (96). It also regulates protein clearance via macrophage phagocytosis (97). Interestingly, it has been demonstrated that Y402H polymorphism of *CFH* strongly reduces its binding affinity for CRP (98) that might disturb protein clearance in the extracellular space of RPE.

There is evidence that inflammation in the pathogenesis of AMD involves macrophage recruitment (99). The macrophages may have a dual role in the complement system regulated protein clearance. It is well known that macrophages represent a heterogeneous group with a spectrum of phenotypes and activities (100-101). However, macrophages may be divided into two main subtypes of macrophages: the pro-inflammatory M1 macrophages, and the relatively anti-inflammatory M2 cells, which function as scavengers and promoters of tissue remodeling. Therefore, it is possible that M2 macrophages in the early stages perform the beneficial, long-term housekeeping role of cleansing protein deposits accumulating in the sub-RPE space. In contrast, M1 macrophages might cause impaired protein clearance, increased drusenogenesis and induce the chronic inflammatory response and development of AMD (Figure 3). Increased M1 macrophage activity is believed to be a result of disturbed complement system due to gene polymorphisms (102). However, further investigation is needed to clarify the associations of protein clearance system, macrophages and polymorphisms in complement system genes in the pathogenesis of AMD.

10. SUMMARY AND PERSPECTIVE

RPE cell degeneration is a primary hallmark in the pathogenesis of multifactorial AMD. Age-associated alterations in RPE cells involve increased oxidative stress, accumulation of lysosomal lipofuscin and extracellular drusens. A decreased capacity to remove damaged cellular proteins in RPE cells has been strongly implicated in the development of AMD. In addition to conventional lysosomal proteolysis, proteasome- or autophagy-mediated proteolysis assisted by molecular chaperones are interesting pharmaceutical targets i.e. developing drugs to maintain effective clearance systems during the aging process. The complement system provides an extracellular therapeutic target to prevent drusen accumulation and AMD. All of these clearance units seem to work in collaboration and are regulated by specific proteins. Therapeutic targets include finding ways to decrease lipofuscinogenesis and to maintain effective proteasome-, lysosome-autophagy- and complement -mediated clearance systems to prevent RPE senescence and AMD.

11. ACKNOWLEDGEMENTS

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