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REVIEW

Stages, pathogenesis, clinical management and advancements in therapies of age-related macular degeneration

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Abstract Age-related macular degeneration (AMD) is a retinal degenerative disorder prevalent in the elderly population, which leads to the loss of central vision. The disease progression can be managed, if not prevented, either by blocking neovascularization ("wet" form of AMD) or by preserving retinal pigment epithelium and photoreceptor cells ("dry" form of AMD). Although current therapeutic modalities are moderately successful in delaying the progression and management of the disease, advances over the past years in regenerative medicine using iPSC, embryonic stem cells, advanced materials (including nanomaterials) and organ bio-printing show great prospects in restoring vision and efficient management of either forms of AMD. This review focuses on the molecular mechanism of the disease, model systems (both cellular and animal) used in studying AMD, the list of various regenerative therapies and the current treatments available. The article also highlights on the recent clinical trials using regenerative therapies and management of the disease.

Keywords Age-related macular degeneration · Retina · Retinal pigment epithelium · Photoreceptor cells · Drusen

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Introduction

Age-related macular degeneration (AMD), an important cause of permanent visual impairment, contributes to 5% of global blindness. AMD is marked by the accretion of lipid/protein depositions known as drusen in the macula of retina, leading to progressive damage in central vision and withering of retinal pigment epithelial (RPE) layer, gradually causing complete loss of sight in advanced stage (Fig. 1) [1]. As conferred through several studies on the physiological and functional changes in RPE during AMD including, mitochondrial DNA damage [2], accumulation of lipofuscin [3] and altered expression of RPE structural proteins [4]; it has been observed that mainly the epithelial monolayer cells are affected with advanced age. The RPE cells show marked significance in sustaining retinal homeostasis majorly through its role as

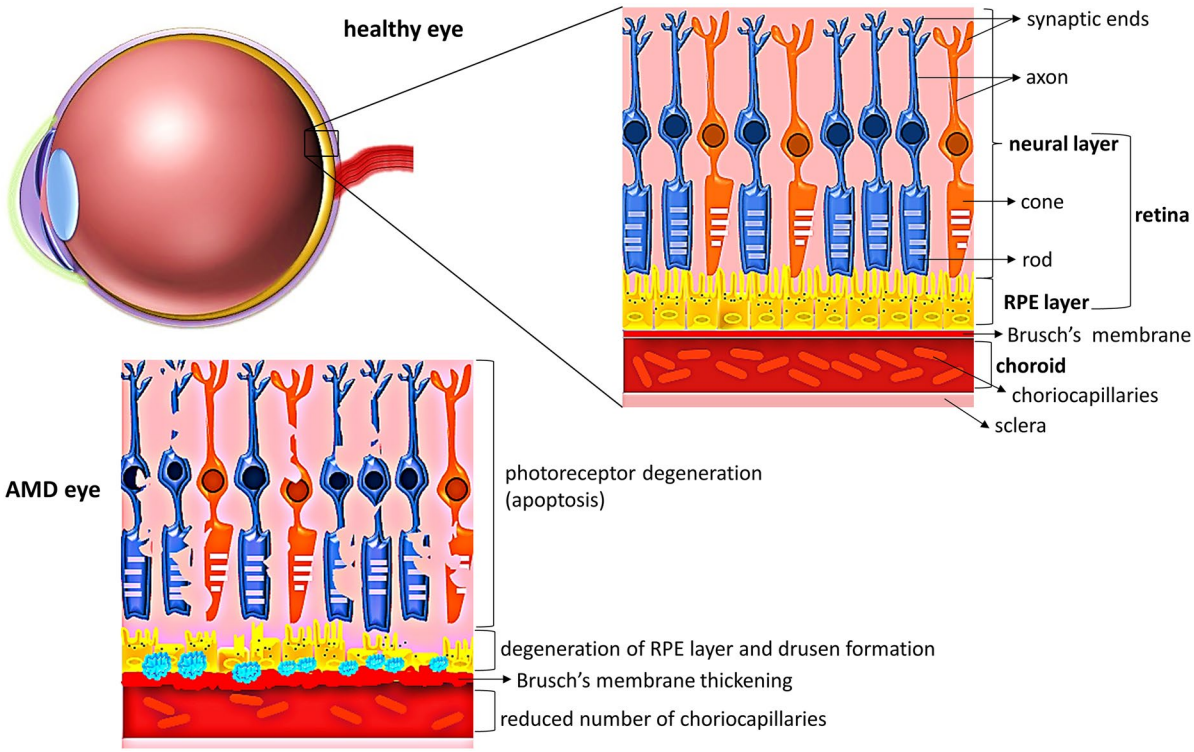

Fig. 1 Diseased versus healthy eye: AMD versus normal eye, featuring the undergoing changes in the neural and retinal layers

a retinal blood barrier, transporter of metabolic products and ions from subretinal space into the blood, as well as a source of angiogenic growth factors release [5, 6] [e.g., transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF)]. It also delivers nutrients derived from the blood to the photoreceptor cells, conducts the phagocytosis of photoreceptor outer segment [7] and absorbs light. However, RPE's constant exposure to light makes it highly vulnerable to oxidative stress that may overtime damage the cellular tight junctions eventually disrupting the retinal blood barrier [8].

Being an age-related progressive disease, multiple risk factors contribute for the development of AMD, which involves both genetic and environmental factors. Complement pathway genes including complement factor H (CFH), complement C2, C3, CFB, toll-like receptor 3 (TLR3), toll-like receptor 4 (TLR4) and ARMS2/HTRA1 are correlated with AMD [9, 10]. Any dysfunction among the immune components such as CFH and TLR3, that are detected in the drusen can lead to apoptosis of RPE and photoreceptor cells resulting in retinal degeneration [11–13]. Environmental and lifestyle susceptible factors including alcohol consumption, smoking, antioxidant intake, hypertension and body mass index (BMI) are also known to induce significant risk on the onset of the disease [14].

An estimated risk of AMD is suspected to reach 288 million by 2040 [15–18] and the disease prevalence increases from 2% for those aged 50–59 years, to nearly 30% for those over the age of 75 years [19]. Asia Pacific alone accounts for more than one-third of the macular degeneration cases [20]. However, Europeans have a higher prevalence of advanced version of AMD, geographic atrophy, (1.11%) than Africans (0·14%) and Asians (0.21%) [18]. "Dry" AMD (also known as geographic atrophy) is a chronic disorder and is characterized by conflux regions of degenerated RPE cells. In contrast, "Wet" AMD affects only 10–15% of AMD patients, however can rapidly progress into blindness if untreated [21, 22]. Gender is another important factor in AMD prognosis; as in high blood pressure, overweight and obesity are associated with late AMD in women only [23]. Women are

potentially at higher risk in developing wet AMD compared to men [24].

Distinctive stages of AMD

AMD is classified into three stages: early, intermediate and late stage. Early stages of the disease show slow progressive painless thinning of the retina (atrophy) caused by an overall loss of neuronal cells due to advanced age and consequently goes unnoticed until later appearing at a severe stage. As the delicate tissue of the retina starts breaking down, the photoreceptor cells (rods and cones) needed for perceiving image also wearies, as a result vision is blurred. Early and intermediate stages of the disease, commonly referred as Dry AMD are distinguished mainly by drusen size (found both internal and external of RPE) [25, 26], changes in the RPE pigments (lipofuscin and melanin) and the degree of loss in vision [27]. Dry AMD shows inflammation and have macrophages and active microglia cells abundant in the atrophic region [28, 29]. It also manifests characteristic degeneration of RPE cells such as loss of melanin and accumulation of lipofuscin pigment, withering of RPE microvilli and disorientation of basal infoldings [30]. Dry AMD is as well associated with photoreceptor cell death, damage of the Bruch's membrane along with manifestation of few small extracellular "hard" drusen aggregates which are otherwise degraded in healthy adults, is normal with advanced age. Nevertheless, an increase in size and number of such aggregates in the macula, characterized as "soft" drusen with large cluster of undefined edges is the prognosis of an early dry AMD, which can be readily detected by funduscopy. This technique relies on the variations in pigmentation caused due to lipofuscin and lipid–protein accumulations in the retinal fundus as a consequence of drusen inflation [31–33]. Late-stage dry atrophic AMD displays blind spot in the visual field, conferring to vision loss. About 65% of the patients with late-stage dry AMD can develop wet AMD, characterized with the appearance of new unusual leaky blood vessels that causes retinal edema, subretinal hemorrhage around the macula, resulting in blurry vision, and consequently interfering with retina's function leading to vision impairment or blindness [34]. However, early and intermediate stage AMD with leaky chorio-capillaries is not uncommon [35, 36]. Another late-stage form of AMD associated with chronic deficiencies in neuronal photoreceptor, RPE, vascular cells and Bruch's membrane is called geographic atrophy (GA), which appears in at least 50% of patients with late AMD [37]. GA is characterized by patches of degenerated RPE regions that are even observed to develop in patients with wet AMD; however, they are not mutually exclusive [38]. The recent Age-Related Eye Disease Study (AREDS) on AMD elucidate negligible possibility of the disease manifestation when few small-sized drusen appear in patients. However, if many small drusen to few or many medium-sized drusen appear in one or both the eyes with occasionally one or more large-sized drusen, they can be indicative of early stage to intermediate stage AMD. In the advanced stage of AMD, the central vision is impaired such as blurry or wavy areas due to damaged blood vessels and photoreceptors cells [39, 40] (Fig. 2).

Mechanisms of AMD: initiation and pathogenesis

The advancement of AMD is marked by the degeneration of monolayer RPE, which is incompetent to regeneration, alongside subsequent loss of adhesion junctions and physical separation with neural retina. RPE acting as a blood–retinal barrier tightly regulate the exchange of metabolites between the neural retina and the chorio-capillaries, and its secretion of growth factors including pigment epithelium-derived factor (PEDF) and VEGF is crucial for the protection of photoreceptor cells and angiogenic response, respectively. However, with age, compromised functions in autophagy can lead to increased amyloid deposits in RPE cells resulting in their death and permanent physical separation of photoreceptor cells, consequently interrupting the photo transduction pathways leading to visual impairment [41]. The physical detachment of the photoreceptor cells from RPE layer also causes their subsequent death, inflammation and vascularization further aggravating the disease condition. Detailed molecular mechanisms leading to AMD disease initiation and progression are discussed below.

Drusen formation in AMD

Drusen and constituents In its earliest stages, AMD is perceived as drusen [42] and their formation may be linked to inflammation (Fig. 3) [43, 44]. This hypoth-

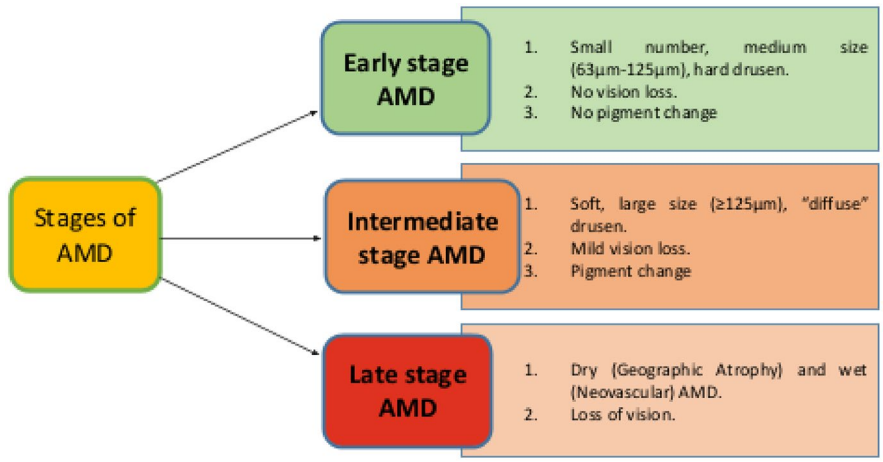

Fig. 2 Distinct stages of age-related macular degeneration (AMD)

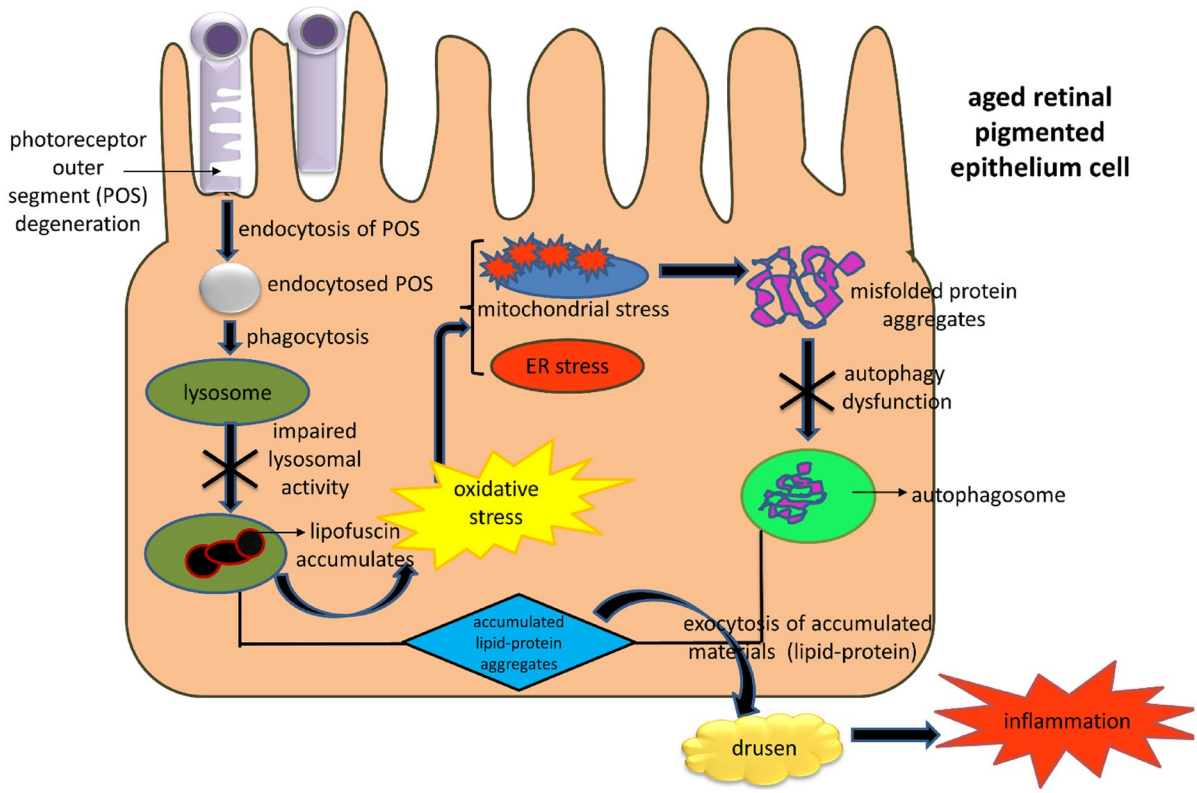

Fig. 3 Schematic representation of the steps involved during drusen formation and inflammation in AMD RPE cell. The figure depicts the process of lipid–protein aggregates deposition, which can lead to drusen and inflammation. In the process of POS phagocytosis by RPE, the endocytosed material is digested within lysosomes. However, with age, dysregulated lysosomal function and decline in lysosome enzyme activity compromise the digestion process resulting in lipofuscin formation. This event subsequently increases oxidative stress within the cell which consequently induces stress conditions in mitochondria and endoplasmic reticulum, leading to misfolded protein aggregates and dysfunctional autophagy linked to impaired protein clearance in RPE. The disturbed clearance of toxic accumulates in aged RPE cells trigger inflammation and the AMD-associated extracellular drusen formation

esis stems from the observation that drusen hub several mediators of inflammation (e.g., C-reactive protein, adducts of the carboxyethylpyrrole protein and immunoglobulins) [43, 45–47], complement factors (complement factor H) and proteins (C3, C5, C6, C7, C8, C9) [44, 48] in addition to membrane-bound complement inhibitors (complement receptor 1—CR1, i.e., CD35) and (membrane cofactor protein—MCP, i.e., CD46) [49, 50]. Based on immunohistochemical analysis of both hard and soft drusen, a wide range of molecules have been detected that are locally synthesized within the RPE, retina and choroidal cells. These include human leukocyte antigen—DR isotype, apolipoprotein E, amyloid A, vitronectin and complement factors 5 and 9. In addition to such various components, the significant localization of both immunoglobulin and C5b-9 indicates the potential role of immune complex activation in the biogenesis of drusen [38].

Drusen as well accumulate lipofuscin (also known as "age pigment"), advanced glycation end-products (AGEs), high level of oxidized low-density lipoproteins and oxysterols which collectively can cause RPE disintegration [51–53]. In addition to lipid molecules (e.g., phospholipids and glycolipids), cholesterol and carbohydrates, drusen also harbor various proteins in significant amounts which include apolipoproteins, vitronectin, clusterin, ubiquitin, fibronectin, integrins, to name among many others [52, 54]. Over time, drusen may push through the RPE cells and disrupt the photoreceptors, causing blind spots in the central vision. Although these blind/blank spots go unnoticed by naked eyes, can be clinically detected as the drusen enlarge eventually causing distorted image formation in the retina.

Reactive oxygen species (ROS) and inflammation leading to drusen formation Wear and tear of day-to-day functions including long constant periods of light exposure, normal visual cycle metabolism and phagocytosis of photoreceptor outer segment (POS) by RPE generates oxidative stress and excess reactive oxygen species (ROS) production, leading to increased inflammatory cytokines release that account for the chronic inflammation and drusen development [55]. Augmented ROS production coupled with oxidative stress plays a pivotal role in AMD pathogenesis. Given the fact that retina has the highest oxygen consumption due to photoreceptors high metabolic activity [56], the polyunsaturated fatty acids (PUFA) (e.g., phosphatidylcholine)-rich photoreceptors cell membrane are readily oxidized generating peroxides and organic radicals in addition to carboxyethylpyrrole and 4-hydroxy-2-nonenal, which form adducts with proteins and are accumulated in the outer retina and in drusen [57–59]. Subsequently, these newly modified lipoproteins are highly active in promoting nonreactive molecules into epitope-like structures that inevitably induce immune recognition and inflammation [60, 61]. In aged RPE, the digestion of oxidized PUFA is disparaged, and as a consequence, it gets deposited in the form of lipofuscin in drusen. Being a chromophore, lipofuscin absorbs high energy photons, which evokes its photooxidation-generating highly reactive *N*-retinylidene-*N*-retinylethanolamine (A2E) [62–64]. Subsequently, A2E upon blue light excitation generates singlet oxygen and superoxide, which collectively with A2E escalates ROS generation and RPE damage in the retina [65].

Oxidative stress, on the other hand can jeopardize natural function of RPE to transport nutrients and ions across choriocapillaris and photoreceptors, exposing the cells to ionic changes that can activate inflammasomes [7, 66]. Several other cellular functions induce the production of inflammatory factors, including activation of pathogen- and damage-associated molecular patterns via pathogen recognition receptors [e.g. toll-like receptors (TLRs), receptor for advanced glycation end products (RAGEs) and NOD-like receptors (NLRs)] by their corresponding ligands [e.g. TLRs recognize elastin, hyaluronic acid and fibronectin and secreted heat shock proteins (HSPs), NLR's sense oxygen radicals, ultraviolet B and potassium (K+) efflux and nuclear factor kappa B is activated with RAGE] [66–75]. Incomplete degradation of phagocytosed POS is linked to the formation of lipofuscin in RPE cells [76], and it is notable that advanced glycation reactions play an important part in the lipofuscin formation [77]. The oxidized lipoproteins bind to RPE cell via CD36 and lectin-like oxidized lipoprotein receptor 1, activating monocytes and macrophages to secrete cytokines (IL-8) and growth factors (TNF-alpha) [78–80]. With growing age, the accumulation of lipofuscin on an account of poor lysosome clearance function in RPE reflects the formation of highly reactive adduct known as advanced glycation end products

(AGEs) to concentrate within drusen as a product of protein (e.g., apolipoprotein E, amyloid and vitronectin)/lipid modification in the course of aging [81–83]. These adducts binds onto the extracellular receptors on RPE [TLRs, RAGEs and AGE receptors (AGERs)], the course of which follows inflammatory signal activation within the RPE and promotes inflammation [84]. While AGEs are endocytosed and removed by macrophages [85], a failure in macrophage recruitment may consequently lead to accelerated retinal tissue damage [84].

Lysosomal and autophagy dysfunction

Besides oxidative stress induced by lipofuscin sensitization of RPE cells to visible light, environmental strain and POS phagocytosis can also promote aberrant increase in ROS production which may lead to mitochondrial dysfunction (Fig. 4). As a consequence of lipofuscin-containing vitamin A-derived fluorophores, it inhibits mitochondrial respiration leading to its dysfunction, hereby promoting protein misfolding [86–88] and generating metabolic deficiency within RPE [87, 89, 90]. Different cellular processes, including HSPs/molecular chaperones, ubiquitination/

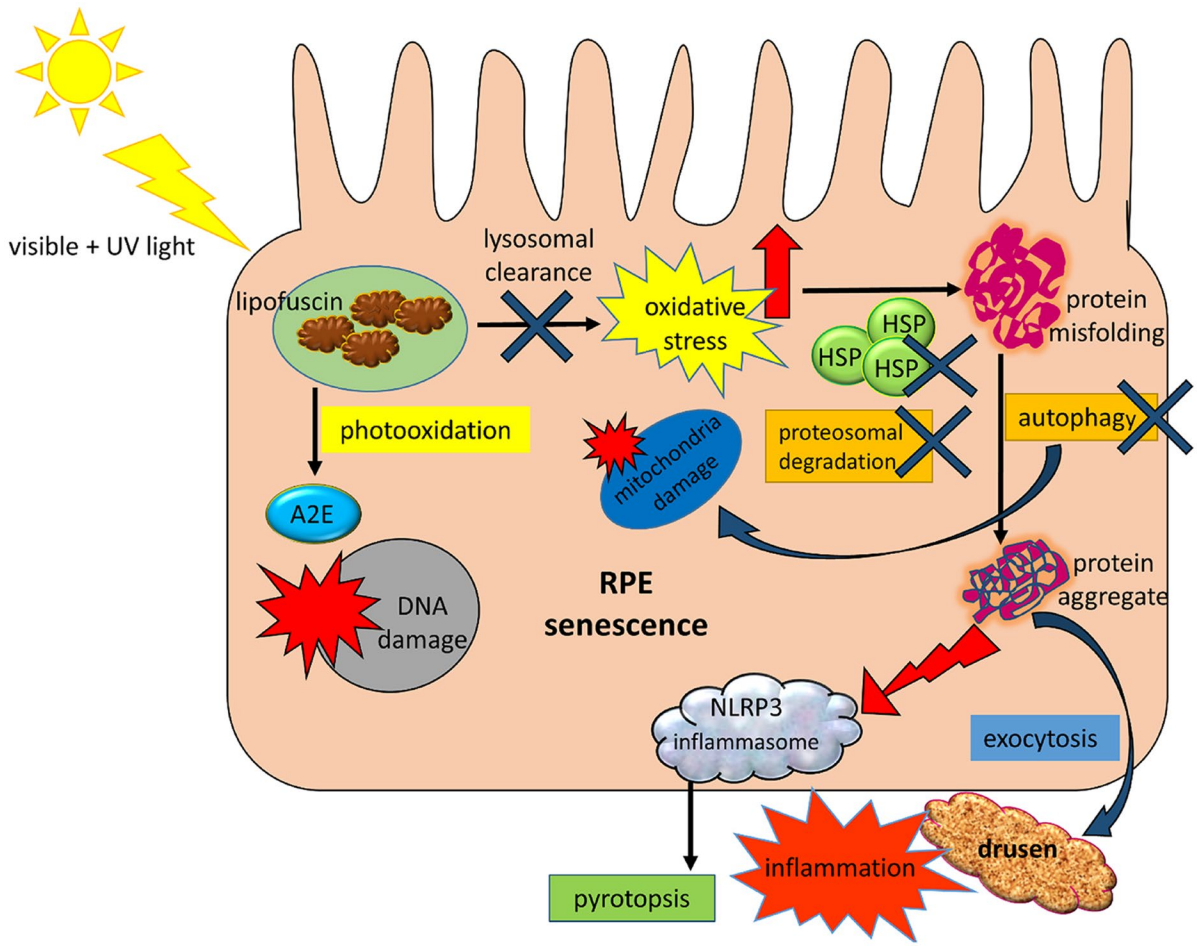

Fig. 4 Schematic representation of RPE senescence in AMD. Constant exposure of RPE to light induces increased oxidative stress and lipofuscin photooxidation. This renders detrimental effects on RPE such as A2E production which causes DNA damage. Mitochondrial damage increases due to decrease in autophagy. Impaired lysosome function results in protein misfolding and age compromised activity of chaperons (HSP) and proteasome damage the protein repairing process which in addition to dysfunctional autophagy forms protein aggregates. Protein aggregates are exocytose as drusen, and NLRP3 inflammasome is activated

proteasomal degradation and autophagy, are involved in clearing the damaged protein overload [91], which ensures cell's survival during oxidative stress condition [92].

In aged RPE cells, the capacity to neutralize ROS weakens due to reduced antioxidant production, poor ability to repair DNA or protein damage and disturbed proteolysis [93, 94]. As a consequence of detrimental concentration of ROS deposition, the cellular proteins are damaged leading to harmful protein aggregation. Lipofuscin is one consequence of this protein aggregates conjugated with lipids since oxidized PUFAs from the POS are not efficiently digested in lysosomes of aged RPE cells [95–98], which consequently induce oxidative stress, ultimately evoking further protein misfolding. Although HSP are available to combat such stress response generated in the cell [92], eventually its capacity also wearies, wherein autophagy fortifies its role in protein degradation [99].

Autophagy, being a lysosome-mediated natural cellular process for clearing damaged cellular substrates, is in particular activated during cellular stress conditions (e.g., oxidative stress, unfolded protein response or inflammation) [100, 101]. The crucial step in autophagic clearance is the fusion of autophagosome with lysosome, a process which is regulated by proteins Rab7, LAMP-2A and soluble *N*-ethyl-maleimide-sensitive factor attachment protein receptors [102]. Such proteins increase the permeability of lysosome membrane and upregulate the membrane-pore openings, promoting the fusion of the two organelles content [103]; thereafter the lysosome proteases (e.g., cathepsins D, B and L) degrade the enclosed cargo proteins [104, 105]. However, during oxidative stress, the enzyme activity of the proteases is decelerated by oxidized lipoproteins [106]. Subsequently, the lysosome function is impaired, which eventually results in decreased autophagy flux and may lead to RPE cell degeneration and AMD development [107]. Degraded autophagy function can also ensue from lipofuscin accumulates that incline to suppress lysosomal functions resulting into impaired autophagy [92]. Lipofuscin deposits once formed are hard to degrade, and upon photooxidation produces A2E [92, 108]. A2E, a photosensitive generator of oxygen free radicals and superoxide can impose toxic effect on RPE cell functions causing increase in DNA damage and inhibiting proteolysis [64, 109]. Additionally, impaired lysosomal function can stem from the event when chronic A2E accumulates tend to inhibit (vacuolar) V-ATPase (a proton-pump), thereby elevating lysosomal pH. As lysosomal enzymes (e.g., acid hydrolases and proteases) are highly sensitive to pH change, thereby any aberration can lead to its dysfunction causing impaired digestion of phagocytosed POS [96, 97, 110, 111].

Studies conducted showed that normal RPE rapidly induced autophagy after starvation in the absence of insulin growth factor (IGF)-1, whereas AMD RPE failed to increase the autophagic flux, ratio of LC3-II/LC3-I (microtubule-associated protein 1 light chain LC3-I, after lipidation becomes LC3-II), under the same conditions [112], [113], wherein the conversion of LC3-I to the autophagic vesicle-associated form LC3-II is the determinant factor of autophagy flux [114]. The addition of IGF-1, expected to suppress autophagy through activation of the AKT/mTOR signaling in normal cells, did not seem to decrease the ratio of LC3-II/LC3-I in AMD RPE [112]. In spite of accumulation of autophagosomes in AMD RPE, the ratio of LC3-II/LC3-I under starvation revealed reduced autophagy [112]. These observations collectively fortify that autophagy is rendered dysfunctional during AMD.

Retinal and photoreceptor cell death

The inception of photoreceptor cell (PC) breakdown is marked by deposits of cellular debris in the form of lipofuscin/drusen underlying the retinal epithelial layer. This results in gradual and permanent retinal detachment cutting off PC's nutrient supply from the RPE cells and the choroid vessels (choriocapillaris), as so compromising their renewal essential for maintaining vision. The constant shedding of the POS, accompanied by their phagocytosis in the RPE cells is crucial to PCs survival. This phenomenon occurs naturally due to retina's long exposure to visual light stimulus which promotes POS phagocytosis, followed by its lysosomal digestion that consequently induce the formation of superoxide radicals (ROS production) in RPE. Conversely, abnormalities can be seen in various diseases, ranging from early-onset retinal dystrophies, such as retinitis pigmentosa or Usher's syndrome to age-related diseases affecting the central retina, such as AMD [115]. Subsequently, POS phagocytosis generates oxidative stress and these

cellular phenomena are crucially interrelated to lipofuscin formation. The toxic A2E component of lipofuscin [116, 117] inhibits RPE's phagocytic functions, finally leading to RPE cell death [118–120]. Elevated oxidative stress in addition to advanced age is ultimate because that can compromise normal functioning of cellular proteins such as ubiquitin. Ergo, ubiquitinated proteins in RPE do not undergo degradation and resultantly accumulates as aggregates in subretinal space which consequently leads to RPE degeneration [118, 120, 121]. The incompetency of RPE cells lysosomes to completely degrade the digested POS is a critical consequence of advanced age, which encourages the accumulation of nondegradable lipofuscin (vitamin A metabolites) within the lysosomes that can potentially cause RPE damage associated with inflammation [43]. Once damaged, the RPE can secrete growth factors like VEGF, basic fibroblast growth factor (bFGF) and TGF-β that are crucial for neovascularization in AMD [122]. To combat the effect, an endogenous anti-angiogenic growth factor known as PEDF has been studied as a potential inhibitor of VEGF [123]. However, in AMD patients, the PEDF levels in the vitreous have been reported to decrease vividly [124].

Ultrastructural pathology studies have suggested cell death in AMD is predominantly through necroptosis and pyroptosis, while apoptosis may have a minor contribution [125]. Necroptosis is reportedly predominant in RPE cell death associated with dry AMD [126], whereas pyroptosis occurs after the NLRP3 (NLR-with pyrin domains) inflammasome priming by IL-1α or C5a that activates inflammasome and alters the death mode induced by photooxidation from apoptosis to pyroptosis [127], 128. Hanus et al. [129] have reported necrosis in ARPE-19 cells when treated with H_2O_2/tert-butyl hydroperoxide in order to induce oxidative stress; the cells subsequently featured characteristics of necrosis such as depletion in ATP and receptor-interacting protein (RIP3) aggregation. An interaction between apoptosis and pyroptosis was studied using ARPE-19 cell line and primary human RPE cells loaded with lipofuscin, irradiated with blue light [130]. The irradiated lipofuscin-mediated oxidative stress resulted in damage to the lysosomal membrane leaking lysosomal enzymes into the cytosol and eventually causing cell death by apoptosis. Tso et al. in 1996, first demonstrated that apoptosis is active in dry AMD [131, 132]. Further Dunaief et al. [133] described an increase in apoptosis in the inner choroid, RPE, photoreceptors and inner nuclear layer with RPE atrophy.

Cellular and animal model systems to study cell death mechanism

Retinal detachment triggers apoptosis of photoreceptor cells was observed among rat models with an increase in caspase-3, 7, 8, 9 activities [134, 135]. Along with intrinsic mechanism of apoptosis, elevated expression of TNF-α, Fas-L and Fas, that regulate apoptosis's extrinsic pathway [136, 137] were also reported, indicating caspases might not be the sole mediators of cell death post-retinal detachment [138, 139]. The mitochondria–nuclear translocation of apoptosis-inducing factor (AIF) was observed after retinal detachment (RD) in experimental rats [138], mice as well as in human retina [140], providing strong evidence of AIF contribution to RD-associated photoreceptor apoptosis. Poly (ADP-ribose) polymerase (PARP) is regarded as an important factor in the regulation of cellular death in AMD. High concentrations of hydrogen peroxide-induced necrotic cell death, mediated by the activation of PARP1 in human RPE cells in culture, while nicotinamide adenine dinucleotide (NAD+) protected the cells against this effect [141]. Injection of Fas receptor inhibitor, Fas receptor-neutralizing antibody, small inhibitory RNA against the Fas receptor, all have shown to decrease the rate of apoptosis of photoreceptors after retinal detachment [133, 135]. When caspases are inhibited by benzyloxycarbonyl-Val-Ala-Asp (OMe) fluoromethylketone (Z-VAD-fmk), necrosis is induced by RIP kinases, which then regulates photoreceptor cell death post RD, where RIP1 and RIP3 act as mediators of necrosis [142]. To further confirm necrosis form of photoreceptor death, it was studied that in human eyes with RD; there was an increased level of box1 protein, a factor released only from necrotic cells but not apoptotic cells [143]. As both necrosis and apoptosis cause photoreceptor cell death after RD, so it could be an effective way to protect photoreceptor degeneration by simultaneously inhibiting caspases and RIP kinase.

Endoplasmic reticulum (ER) stress-induced apoptosis in retinal cell death has been observed during AMD in both cultured RPE cells and in animal model retinas. Expression of a mutant (R14W) of

carbonic anhydrase IV, a glycosylphosphatidylinositol-anchored protein, highly expressed in the choriocapillaris of human eye, upregulate Bip (ER chaperone that facilitates protein folding and reduces ER stress), protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) and CCAAT/enhancer-binding protein homologous protein, a central mediator of ER stress-induced apoptosis (CHOP), markers of ER stress and the unfolded protein response, is accompanied by apoptosis [144]. Overexpression of Bip attenuates CHOP expression, apoptotic cascade activation and restored the retinal photoreceptor function in P23H rats [145]. Similarly, enhanced ER stress was reported in retinitis pigmentosa induced by the rhodopsin mutation P23H in Xenopus laevis [146] and also in rats [147]. In degenerating rd1 mouse retina, translocation of caspase-12 from the inner segments to the nuclei of the photoreceptors was studied with a further study confirming caspase-12 involvement in rd1 cell death and along with other ER stress-related factors such as GRP78/BiP, EIF2a and PERK [148, 149].

Clinical management

In spite of the progressive loss of vision caused by AMD, currently the prospect of preventing or curing macular degeneration is highly limited. Until recently the only available treatment to seal leaking blood vessels associated with wet AMD was with a laser called laser photocoagulation. Currently, clinical interventions for AMD involve intravitreal injection of anti-VEGF drugs such as pegaptanib sodium injection, aflibercept and the United States Food and Drug Administration (FDA) approved ranibizumab. However, these treatments do not target the underlying degeneration inherent in wet AMD, and there is high rate of recurrence when such treatments are discontinued [150]. In case of surgical removal of the choroidal neovascular membrane, vision improvement is often limited due to the previous damage already caused within the RPE, and the procedure might as well risk the amputation of remaining RPE and photoreceptor cells. So far treatment administered for dry AMD encompasses allogeneic transplantation of RPE cells derived from human fetuses, nevertheless this was usually observed to be risked with graft rejection [151, 152]. Transplantation of RPE cell sheet is an uprising technique to treat dry AMD, still it's invasive and complex surgical procedure that is associated with a high risk of massive hemorrhage and retinal detachment. One of the commonly used treatments for wet AMD is photodynamic therapy (PDT), which is based on the delivery of a photosensitizer to the choroidal neovascularization (CNV) site via a liposomal formulation of verteporfin [153], which in combination with anti-VEGF is known to maintain visual function during CNV [154]. Besides, AREDS studies also assessed the use of vitamins in the progression of AMD treatment [155]. Current AREDS studies suggest that omega-3 fatty acids, antioxidants and zinc might reduce AMD or may even restrict the rate of its progression [156]. The researchers of AREDS investigated a formula of antioxidant vitamins C and E, beta-carotene and zinc (known as the AREDS formula) to be orally administered on AMD patients. However, it was observed to initiate the risk of lung cancer in patients addicted to smoking [157]. To counter the side effects, the formula was later modified in 2006 and renamed as AREDS2 design, that may test the effectiveness of the study by administering omega-3 fatty acids or lutein and/or zeaxanthin [156, 158].

Current treatments

Neuroprotective treatments and gene therapy as can be aided to prevent initial stages of degeneration are however found to be inefficient when comes to treating the later stage of the disease. In cases of severe vision loss, retinal prosthesis devices were approved by FDA although it provides resolution below the level of functional vision and use external sensors for light detection [159–162]. Contrariwise, targeted replacement of photoreceptors can overcome such challenges and similar to RPE replacement, uses both embryonic stem cells (ESC) and induced pluripotent stem cell (iPSC)-derived in vitro cultures in cell replacement therapies [163–167]. The current gold standard in treatment of neovascular AMD has been through the advent of intravenous VEGF inhibitors (e.g., ranibizumab/bevacizumab, aflibercept approved by FDA) [168], though the recovery rate has been estimated to be only 30% in patients [169]. The limitation of this treatment resides for the fact that sustained blocking of VEGF, an essential factor for cell survival, can accentuate to chorio-retinal atrophy.

Regenerative therapy

Photoreceptor replacement During cellular degeneration, it is much easier to replace RPE cells than photoreceptors as the latter clump rather than forming a single layer. Photoreceptors, however, after being transplanted into the retina must connect with the retinal neurons and form synapses in order to conduct their signals. Though this normally occurs in the developing retina, it is much harder to accomplish in an adult retina. Inspired by the early trials of RPE replacement, photoreceptor replacement was initially attempted using full-thickness retinal sheets or patches [170–172]. Later on, direct subretinal transplantation was attempted using undifferentiated retinal progenitor cells, photoreceptor precursors and forebrain-derived neuronal progenitors, which were expected to differentiate into photoreceptors due to the subretinal space microenvironment [173–178]. Various methods are tested for rod photoreceptor cells generation from pluripotent stem cell sources, though it appears to be more challenging to consistently generate high numbers of cone photoreceptors from stem cells in vitro [179–184]. However, encouraging data are emerging on methods to increase the fraction of cone photoreceptor cells generated in culture and transplantation [185–189].

RPE transplantation as a therapeutic model Recently, gene therapy was proven efficient in restoring RPE function [190, 191], yet RPE replacement remains a viable strategy for retinal degenerative diseases. Stem cells are used to replace the damaged RPE cells specifically in atrophy AMD by transplanting ESCs- or iPSC-derived products into the macula, paracentral retina or vitreous cavity so as to restore vision. Subretinal transplantation of human embryonic stem cell (hESC)-derived RPE cells has been reported [180, 192–194]. Similarly, autologous iPSC-derived RPE cells were safely transplanted into an AMD patient without immunosuppression [195]. The advancement in stem cell research, has escalated the possibility of maintaining retinal neurons in vitro [196], followed by the de novo differentiation of retinal neurons from either ESC or iPSC lines [197, 198]. Major strategies for cell transplantation are injection of a suspension of cells (less invasive) and surgical implantation of an RPE monolayer, with or without a supporting membrane. Studies by Carr et al. [185] demonstrated that injection of RPE cells in rat models tend to form clusters and show limited phagocytosis of photoreceptor outer segments. An experiment comparing injection and implantation of hESC-RPE revealed that implanted monolayers survived longer (for at least 12 months) without evidence of tumor formation in immunocompromised rats [199]. A scaffold-free layer of iPSC-RPE, designed for clinical use showed no immune rejection or tumor formation when implanted in a primate model [200]. Furthermore, human clinical trials using suspension injections of hESC-RPE had no uncalled safety issues related to the injected cells [201]. Based on this research, first individual with AMD to ever receive a transplanted layer of autologous iPSC-RPE cells was reported [202].

Biomaterials The use of purified extracellular matrix proteins (such as collagen IV and laminin) differentially influences hESC-RPE growth, pigmentation, barrier function and also improves the production of differentiated iPSC-RPE cells [203]. Bioengineered polymers used as matrices promote the formation of a single layer of polarized RPE cells with specialized apical and basal features, the disruption of the same that is implicated in retinal diseases [204]. Synthetic Bruch's membranes were constructed from fibroin, supported the co-cultivation of RPE cells and microvascular endothelial cells [205]. Biodegradable and biocompatible biomaterials working as curative matrices either individually or along with the cell transplants or drug-loaded matrices, are widely explored. The commonly used polymers are polylactide, polylactide-co-glycolide and acrylic polymers, which can be degraded in vivo to form natural metabolites. Porous poly(ε-caprolactone) (PCL) is biocompatible, helps metabolite transport and improve human fetal retinal pigment epithelium cell function compared with non-porous PCL or porous polyester [206]. Other polymers engineered for RPE transplantation, include parylene [207]. Parylene, a xylene-based hydrocarbon polymer approved for biomedical use and can be engineered with ultrathin regions such that it has permeability similar to Bruch's membrane [208]. hESC-RPE cultured on these ultrathin parylene-C membranes are able to adhere, proliferate, develop polarized monolayers and maintain RPE characteristics [208]. Advanced surgical techniques were developed to implant the parylene substrates into a rat model of AMD, where more than 98% of the transplanted RPE cells survived the

procedure [209]. Future RPE implants might include biocompatible scaffolds that mimic a healthy Bruch's membrane [210].

Nano-therapeutics Nanoparticles (NPs) may be used to deliver drugs for easier (topical) and sustained delivery, reducing the frequency of intravitreal injections. NPs loaded with ganciclovir administered into the vitreous, showed prolonged presence of the drug in the eye with least toxicity manifestation [211]. Inorganic NPs are observed to have anti-angiogenic properties as reported by Kim et al. [212] and Jo et al. [213] in their studies using gold and silicate nanoparticles, respectively, which could be considered as a viable treatment for neovascularization. The use of yttrium oxide nanoparticles (Y_2O_3) as free radical scavengers has been reported to prevent photoreceptor cell death and an alternative treatment for oxidative stress associated retinal degeneration [214, 215]. Therapeutic genes may also be delivered to RPE and photoreceptor cells by NPs. For example, studies reported using murine model of retinitis pigmentosa photoreceptor cells that were treated with CK30PEG10k-compacted DNA nanoparticles, which led to transgene expression in RPE [216]. Also, liposome–protamine–DNA complex was used as delivery system for RPE65 gene in knock-out mice, which efficiently expressed RPE65 gene for a long time [217].

Indeed, NPs were shown to be taken up by RPE cells both in vitro and in vivo [218–221]. Recent studies using NPs as a delivery system for marker gene encoding green fluorescent protein (GFP) into the subretinal space or vitreous of adult mice showed significant levels of GFP expression in photoreceptors and RPE cells [222, 223]. Interestingly, nanoparticles, in advantage to their small size (< 100 nm), when injected in the vitreous can migrate through the retinal layers and tend to accumulate in the RPE cells [219, 220]. For instance, nanoparticles (1–1000 nm, generally 20–300 nm) of liposomes can be developed for sustained release of intraocular drug [224]. Sakurai et al. [225] reported that the size of nanoparticles is correlated with the efficiency of drug delivery in vitreous humor through the study using intravitreous injection of three sizes of nanoparticles (50 nm, 200 nm and 2 μm) in rabbit eyes. VEGF antisense oligonucleotides impregnated with NPs were successfully delivered to ARPE-19 cells and inhibit VEGF secretion and mRNA expression [218]. Also, studies with bFGF-loaded NPs showed significant protection against photoreceptor degeneration in RCS rats due to sustained release of bFGF following intravitreal injection [226]. Nanotechnology-based PDT has been recently tested in laser-induced CNV animal models. The use of a dendritic photosensitizer (dendrimer porphyrin encapsulated by a polymeric micelle) led to a highly selective accumulation of photosensitizer in the CNV lesions, and significantly enhance the efficacy of PDT [227]. These data provide a novel paradigm for the treatment of AMD through dendrimer-based nanomedicine. Hence, the intracellular delivery of molecules by NPs to RPE or photoreceptor cells may open a wide range of therapeutic avenues for AMD.

Conclusion

Regenerative therapies though have addressed the different forms of AMD pathogenesis, there is currently no treatment approved that may completely cure the disease. However, several ongoing trials are aiming to find way to at least cease the progression of early AMD into an even serious stage. Several challenges need to be tackled in order to restore the damaged epithelial cells, neural layers and avert atrophy manifestation. Nevertheless, the progress in clinical translational research in replacement of RPE using stem cells is currently in progress, but the overall advancement in using in vitro models for retina neural cell replacement is still not promising. Although positivity is seen with in vivo models in understanding the effectiveness and safety of new therapies for AMD, the next leap toward clinical translation must be carefully approached and with treatments made available to the patients at financially reasonable level.

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Declarations

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