New Insights into AMD Pathogenesis

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Abstract

Age-related macular degeneration is the leading cause for blindness in the elderly of the industrialized world. The disease cause remains elusive although many risk factors have been identified. Most research efforts are focused on the retinal-pigmented epithelium due to its role in both advanced disease stages, geographic atrophy, and choroidal neovascularization. Here we discuss the role of photoreceptors in age-related macular degeneration pathogenesis. We review data that suggest a possible link between metabolic adaptations in photoreceptors of patients and the effect of these adaptations on the development and progression of the disease. The findings suggest that a change in photoreceptor lipid synthesis contributes to the development and progression of age-related macular degeneration. This change is likely precipitated by an adaptive response in photoreceptors to a nutrient shortage experienced during the early disease stages. Understanding the role of altered lipid metabolism in photoreceptors may provide new avenues for the treatment and prevention of age-related macular degeneration.

Keywords

Photoreceptors · Dry AMD · Geographic Atrophy · Drusen

12.1 Age-Related Macular Degeneration: The Leading Cause of Blindness in the Elderly

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly of the industrialized world [1]. The prevalence of AMD worldwide is increasing every year due to the growing population and the increase in life expectancy. A prediction in 2014 estimated that 196 million people worldwide would suffer from AMD by 2020 and 288 million people by 2040 [2]. In Europe, approximately 67 million people are affected by AMD, a number that is projected to increase by 15% in 2050 [3]. These increases are not only due to a growing population and an increase in life expectancy but also to the fact that early signs of AMD are starting to develop at younger age [4]. One plausible explanation is the excessive use of digital devices in the industrialized world, which has become an inevitable routine of daily life. The light-emitting diode (LED)

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that radiates from screens contains the full spectrum of light, including the short-wavelength high-energy blue light. This more harmful blue LED can cause retinal stress, which manifests as decreased retinal function, increased photoreceptor (PR) cell death, and activation of microglia [5, 6]. Microglia activation has been observed in many retinal diseases, including AMD, although the role of microglia in AMD pathogenesis remains unclear [7, 8].

AMD is a slow progressing macular degeneration disease. While the disease does not only manifest in the macula, once degeneration spreads into the macular region, vision is severely compromised. This is because the macula harbors the cone-rich fovea, which is the area responsible for high-acuity vision in humans. Early-stage AMD patients develop yellow spots scattered around the macula, which are visible by funduscopic examination. These yellow spots, also known as drusen deposits, were later found to be composed of cellular debris that accumulates between the Bruch's membrane (BrM) and the adjacent single-cell layer of retinal-pigmented epithelium (RPE) cells [9, 10]. Drusen are also rich in lipoproteins [11]. In approximately 20% of patients with drusen, the disease progresses to one of the two advanced forms of AMD, exudative and non-exudative AMD. Non-exudative or dry AMD is characterized by the formation of large confluent patches devoid of RPE cells. Since RPE cells support PR function, PR cell death follows the atrophy of the RPE. Because of the focal nature of RPE and PR cell death, the lesions of cell death are also referred to as geographic atrophy (GA). The exudative form of AMD is characterized by choroidal neovascularization (CNV), which results subsequently in leakage of blood fluid into the subretinal space. This disrupts retinal function and leads to loss of PRs. The buildup of fluid in this advanced form has given rise to the conventional nomenclature of "wet AMD." Around 10% of advanced AMD patients have the wet form, which if untreated leads to rapid irreversible vision loss. Current treatments for wet AMD use antibodies or chimeric receptors that bind to the vascular endothelial growth factor (VEGF) thereby neutralizing its action. Patients are injected intravitreally with these anti-VEGF proteins, which act as a sponge by adsorbing excess VEGF in the eye. This reduces the leakage of fluid from these newly formed blood vessels and prevents further growth of new blood vessels. While this treatment is very effective, not all patients respond to it. In contrast to wet AMD, there is no treatment available for dry AMD, which affects the majority of advanced AMD patients (~90%). Vision loss progresses slower in patients with dry AMD; however, the lack of FDA-approved treatments for dry AMD increases the need to find treatment modalities for this more frequent form of the disease.

12.2 AMD Is a Multifactorial Disease

AMD is a complex multifactorial disease with age being the biggest risk factor. Other risk factors including smoking, diet, and genetics have also been implicated in many studies [12, 13]. Despite the identification of over 30 risk factors [14], the underlying cause for disease onset and progression remains unclear. This lack of understanding has complicated the identification of therapeutic targets for the development of treatments. To overcome this hurdle, several animal models have been generated based on genetic and non-genetic risk factor to study their role in AMD pathogenesis [15–17].

Genome-wide association studies have identified over 50 gene variants that are associated with AMD [14]. Among the disease-associated genes, a large number of them are found to be part of the innate immune response, playing an important a role in complement regulation [18–21]. The complement system has been shown to be important in maintaining retinal integrity and function during aging [22]. Histopathological studies with tissue samples from deceased AMD patients revealed the presence of complement factor C5, C6, C8, C9, and CFH in drusen deposits, suggesting an involvement of the complement pathway during disease progression [10, 23]. Additionally, a genome-wide association study found that one of the single-nucleotide polymorphism (SNP)

variants in CFH, CFH Y402H, which has a tyrosine-histidine change at amino acid 402, is strongly associated with AMD [21]. This led to the generation of different mouse models to elucidate the function of CFH in the retina and AMD pathogenesis [21, 24, 25]. In homozygous *cfh* knockout mice, retinal function is significantly reduced at 24 months of age [24]. Other retinal phenotypes seen in aged CFH-deficient mice include an increase in autofluorescent deposits, thinning of the BrM, and disorganized rod PR outer segments (POS), suggesting CFH is required to maintain long-term retinal health [24]. To further elucidate the contribution of CFH to AMD pathologies, humanized CFH transgenic mouse lines were generated, which express the full-length human *CFH* allele with either a tyrosine or a histidine at amino acid 402. These lines were then crossed into $cfh^{-/-}$ mutant background [25, 26]. Consistent with the histidine change playing a role in AMD pathogenesis, mice expressing the normal human CFH allele showed partial recovery of retinal function and morphology, while mutant CFH allele-expressing mice displayed less rescue of the pathologies [25]. Findings in animal models on the role of complement in the retina have led to the development of drugs that target various components of the complement system [27]. Several clinical trial studies have been initiated, and some have already been completed; however, most of these trials show only a mild therapeutic effect, if at all, in a subgroup of patients with late-stage AMD [27]. While the data suggest that complement plays a critical role in AMD pathology, its contribution to disease progression requires further investigation.

Another large group of genes that increase disease risk is associated with cell metabolism, in particular lipid metabolism. Lipid metabolism has been shown to play an important role in AMD pathogenesis [28, 29]. In aged individuals, there is a progressive buildup of lipids in the BrM, which results in a slow deterioration of the BrM structure [30]. The buildup was eventually proposed to be driven by RPE-derived apolipoproteins, such as ApoB and ApoE, creating an extensive oil barrier at the BrM, which is thought to be the precursor of the pathological basal linear deposits (BlinD) and soft drusen [11]. Histopathological studies also confirmed the presence of apolipoproteins in different types of drusen [10]. In addition to the epidemiological and histological studies, genome-wide association studies on *APOE* isoforms discovered that epsilon 3 and 4 alleles may decrease disease risk for AMD, while epsilon 2 allele shows slightly increased disease risk for AMD [31–33]. Interestingly, studies with transgenic mice that express the human *APOE* alleles and are fed a high-fat diet show that the *APOE* 2 and 4 alleles increase disease risk [34]. In particular, mice expressing the *APO4* allele exhibit AMD-like pathologies including, thickened BrM, dursenoid deposits, hyperpigmentation, and CNV [34]. While these human and mouse studies are contradictory [31–34], they suggest that apolipoprotein mediated lipid circulation in the retina may play a role in disease pathogenesis.

Elucidating the underlying disease cause for AMD has remained complicated, even though many risk factors have been identified and many animal models based on these risk factors have been generated. This may be due in part to the fact that none of the mouse models generated fully recapitulate the entire disease spectrum seen in patients with AMD [15–17]. One explanation for this discrepancy could be that mice do not have a macula with the cone-rich foveal structure, making it hard to replicate all AMD features. Another explanation for this problem could be that there are other unidentified factors that contribute to the underlying disease mechanisms.

12.3 Photoreceptors in AMD Pathogenesis

PRs are among the most metabolically active neurons in the body. The reason for this is that PRs require large quantities of energy to maintain their resting membrane potential as well as to sustain the daily regeneration of the shed POS [35]. The highly metabolic PRs may shift their metabolism during periods of nutrient depriva-

tion to adapt and survive longer. Indeed, the secondary cone death phase in retinitis pigmentosa is characterized by an adaptive response of cones to the nutrient depravation brought about by the death of rod PRs [36–38]. Enhancing this adaptive response by further activation of the mammalian target of rapamycin complex 1 (mTORC1), a kinase that regulates cell metabolism in response to nutrient shortage, prolongs cone survival in an otherwise nutrient-deprived environment [36–38]. In the retina, RPE cells function to maintain PR homeostasis [39]. They reduce the oxidative stress PRs experience due to light exposure by phagocytosing the tips of the POSs, which removes reactive oxidative species (ROS) as well as oxidized lipids and proteins generated during phototransduction [40]. Phagocytosed POSs that are packaged into phagosomes fuse with the RPE lysosome to become phagolysosomes, which leads to the degradation of the oxidized material. Inefficient degradation has been linked to lipofuscin accumulation in the RPE and an increase in RPE oxidative stress, both hallmarks of AMD [41]. Increased oxidative stress in the RPE has also been linked to mitochondrial dysfunction [42]. This has been proposed to alter the energy flow of the PR-RPE ecosystem [39, 43] in AMD patients. Because of the metabolic interdependence between PRs and RPE cells [39], any perturbation of the ecosystem may affect the health and survival of both cell types [42–45]. While it remains unclear what initiates RPE cell stress in AMD patients, the existence of mitochondrial dysfunction in RPE cells of AMD donor eyes [43] and the presence of a lipid wall at the BrM [11] both suggest that PRs of AMD patients are nutrient deprived, like cone PRs in retinitis pigmentosa patients [36, 38].

Most research efforts on understanding AMD pathogenesis have focused on the RPE due to the evident pathology of RPE atrophy (referred to as GA) and the breakdown of the RPE–choroidal blood barrier during CNV. PRs are generally considered as an affected bystander in the pathogenesis of AMD. However, several lines of evidence also suggest a more active role of PRs in AMD pathogenesis. For example, the macular translocation procedure, which was developed to save the cone dominant fovea by relocating the fovea exposed to dying RPE cells to another healthy RPE region, revealed that PRs may contribute to RPE stress and the late disease stage of GA. Patients whose retinas were rotated redeveloped GA in the area where the macula was placed [46, 47]. The authors of these studies attributed the development of GA in the newly translocated area to the high metabolic needs of the foveal cones. Similarly, extensive histopathological studies have found that soft drusen develop preferentially where the foveal cones are, while another type of drusen that accumulates between the PRs and the RPE, the subretinal drusenoid deposit, develops preferentially in perifoveal region, where rod density is the highest [48, 49]. The authors of these studies attributed the distribution of the different drusen types to the difference in the metabolic needs of rods and cones. Both studies suggest that PRs could contribute to the late as well as early disease stages of AMD through their different high metabolic needs. However, this would mean that PR metabolism would have had to change, since not all aged individuals develop AMD. Alternatively, a uniform sick RPE in AMD patients may also explain these findings without changes in PR metabolism, because of the metabolic interdependence of PRs and RPE cells. While we know that RPE cells in AMD patients display signs of oxidative stress and mitochondrial dysfunction, little is known about metabolic adaptations in PRs of AMD patients that are predicted to occur due to presumed nutrient deficiency. Similarly, it remains unknown if altered PR metabolism can lead to AMD pathologies.

12.4 Mimicking the Metabolic Adaptations Seen in PRs of AMD Patients Leads to AMD-Like Pathologies in Mouse

To determine if PR metabolism is altered in AMD patients and to study any effect of these changes on AMD pathogenesis, we used immunohisto-

chemistry to identify expression changes of metabolic genes in human tissue samples, and mouse genetics to mimic these changes and study their effects on the eye [50, 51]. We used mouse to mimic the human changes since both human and mouse have a similar rod-to-cone ratio, albeit mouse does not have a fovea. The density of PRs and the phagocytic stress of RPE cells in the central mouse retina is similar to the peripheral part of human macula. This allows testing the contribution of altered PR metabolism on RPE stress under similar conditions that may exist in humans [52]. Finally, AMD is not a fovea-only disease, since GA occurs often outside the fovea or the macula.

To determine if there are any metabolic adaptations in PRs of AMD patients, we looked at the expression changes of two key metabolic enzymes, pyruvate kinase M2 (PKM2) and Hexokinase-2 (HK2). We knew from our studies on retinitis pigmentosa that PRs respond to a reduction in glucose availability by increasing the expression of genes that improve glucose retention and redirection into the anabolic pathway, which helps maintain POS growth [36–38, 53, 54]. We assumed that the nutrient deprivation would be mainly caused by a shortage of glucose since both lipid buildup at the BrM and the mitochondrial dysfunction in RPE cells seen in AMD patients would reduce glucose flow from the RPE to PRs [11, 43]. We found that both genes were expressed at much higher levels in PRs of AMD patients [50]. This suggested to us that PRs in AMD patients are indeed nutrient deprived. In mouse, this nutrient deprivation is accompanied by increased activity of the kinase mTORC1, which regulates metabolic adaptations including the expression of genes like HK2 and PKM2 [36–38, 53, 54]. To determine if such metabolic changes can contribute to AMD, we constitutively activated the kinase mTORC1 in PRs of wild-type mice, by removing the negative regulator of mTORC1, the tuberous sclerosis complex 1 (TSC1) protein. We found that increasing mTORC1 activity in either rod or cone PRs resulted in AMD-like hallmarks, including increased accumulation of apolipoproteins, ApoB and ApoE, and complement system component CFH, at the BrM of all aged mice [50]. Like the distribution of soft drusen in humans, the formation of drusen in mouse was dependent on the activation of mTORC1 in cones [50], suggesting a clear distinction between the effects of cone and rod metabolism on disease progression. Interestingly, around 20% of the mice with activated mTORC1 in rods developed GA, while only 5% developed neovascular pathologies. All these advanced pathologies developed with age at a frequency that is similar to the percentage of early-stage AMD patients that progress to advanced AMD [50]. Advanced pathologies were also seen if mTORC1 was activated only in cones, albeit at later timepoints. Consistent with that, activation of mTORC1 in both PR cell types led to an earlier onset of advanced pathologies when compared to activation in rods alone [50].

To determine to which extent changes in the metabolic ecosystem between PRs and the RPE [39] are responsible for the development of these AMD-like pathologies, we removed simultaneously the TSC1 protein and HK2 from PRs [51]. These mice have constitutively activated mTORC1 in their PRs but are unable to increase glucose metabolism. The production of lactate and NADPH in these retinas is like that of wild-type mice. Despite these changes, mice still developed the same AMD-like pathologies as seen with removal of TSC1 alone. The data suggest that the metabolic adaptations that are driven by increased mTORC1 activity and cause AMD-like pathologies are independent from the changes in the glucose and lactate exchange between the retina and the RPE [51]. This indicates that gene expression changes, which are driven by mTORC1 and cause disease onset and progression, are unrelated to genes that regulate glucose metabolism.

In human and mouse, RPE cells interact with ~30 to 50 POSs. The shedding of POSs and the phagocytic activity of RPE cells is regulated by the circadian rhythm [52, 55]. In mice with activated mTORC1 in PRs, we found that RPE lysosomal activity is disrupt as POS clearance is significantly delayed. Thus, the metabolic adaptations in PRs may be a stressor for RPE cells. Interestingly, rod POS clearance was also delayed

when mTORC1 was only activated in cones [50]. Moreover, the delay was still apparent when mTORC1 was activated and HK2 was deleted from PRs [51], suggesting that the stress experienced by RPE cells is independent from changes in glucose usage in PRs. This suggests that despite the metabolic coupling between PRs and RPE cells [36, 39, 51, 56], and the changes seen in RPE [43] cells and PRs [50] of AMD patients, the disease-causing changes in humans are likely changes that accompany these metabolic adaptations.

12.5 Importance of Lipids in PRs and AMD Pathogenesis

The POS has a high content of membrane proteins and lipids in order to support the machinery for phototransduction [57, 58]. In rods, membrane lipids and proteins form individual membrane discs that are packed with the components of the phototransduction machinery. It has been proposed that a single-rod POS holds around 1000 membrane discs stacked on top of each other in order to decrease the probability that light passes through the retina undetected [59, 60]. These membrane discs are enriched in phospholipids, in particular in phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS) phospholipid [61].

PR phospholipids have several important functions that help maintain proper retinal homeostasis. They stabilize the phototransduction complex and help with retinoid transport across the membrane [58, 62]. Phospholipids also regulate RPE cell phagocytosis [63]. The shed POS [40] requires a receptor-ligand binding interaction with the RPE. Oxidized PC phospholipids on the POS generated during phototransduction serve as "engulfment signal" for receptors expressed on RPE cells [63]. Together, the data suggest that phospholipids are crucial for proper POS and RPE function.

mTORC1 is a key kinase that regulates various metabolic pathway including lipid synthesis [64]. Thus, the metabolic shift induced in our mouse model by activation of mTOC1 in PRs may have contributed to RPE cell stress through altered phospholipid synthesis in PRs. Such change could be a contributing factor in the development of AMD-like pathologies seen in our mouse model. Indeed, we found in mice with activated mTORC1 in PRs a significant reduction in two types of phospholipids, specifically PC and PE phospholipids, which have two docosahexaenoic acid (DHA) side chains [50].

DHA is an omega-3 polyunsaturated fatty acid that is highly enriched in the retina [65]. In general, POSs are rich in polyunsaturated fatty acids, to maintain a high mobility of the phototransduction proteins in the disc membrane [61]. However, this high level of unsaturated fatty acids may also contribute to retinal stress through lipid peroxidation-induced modification of proteins [66]. Once shed, these modified proteins increase lysosomal dysfunction in the RPE, lipofuscin accumulation, and AMD disease risk [66]. A reduction in polyunsaturated double DHA-containing PC and PE phospholipid in our mice may thus represent an adaptative response of PRs that experience nutrient stress in order to prevent further oxidative damage to both the retina and the RPE [67]. Dominant rhodopsin mutations in rats result in reduced DHA accumulation in POS, suggesting that a PR intracellular stress response may reduce DHA incorporation to prevent further lipid peroxidation-induced modification of proteins [68].

DHA has also been shown through many studies to play a crucial role in maintaining retinal integrity [69, 70]. Over 15 epidemiological studies linked dietary consumption of DHA to a reduced disease risk for AMD [28, 29, 71–73]. Surprisingly, the age-related eye disease study 2 (AREDS2) found no correlation between dietary DHA supplementation and AMD prevention [29, 74]. Weaknesses in the study design have been proposed to explain these conflicting results [29]. To further elucidate the role of DHA, we fed our mice with reduced di-DHA PE and PC lipids a DHA-enriched diet that had 5X the amount of DHA used in the AREDS2 study. The amount was based on a smaller study conducted in humans that that found a protective effect of DHA on disease progression [75]. We found an

overall improvement of AMD-like pathologies in our mouse model when mice were fed a DHA-enriched diet [50]. Neither GA nor CNV were observed in DHA-fed mice, and the accumulation of apolipoproteins at the BrM was also reduced [50]. Strikingly, just 2 weeks of DHA feeding in 6-month-old mutant mice was sufficient to recover a severely impaired RPE phagocytic activity [50]. When we reprofiled the retinal lipids to measure the increase in these di-DHA PE and PC lipids after DHA feeding, we found no change in mice with activated mTORC1, while in wild-type mice, di-DHA PE and PC levels were increased [50]. The results suggest that DHA supplementation improved RPE health and AMD-like pathologies by acting directly on the RPE. RPE cells normally recycle a certain percentage of the DHA from shed POS during phagocytosis and deliver it back to PRs for POS renewal [76]. Some of the DHA taken up by RPE cells during phagocytosis of the POS is also converted into neuroprotection D1 (NPD1) to prevent oxidative stress-induced apoptosis, reduce inflammation and cell damage, and improve autophagy [77–81]. Exactly how the reduction in PE and PC di-DHA lipids in our mouse model affects disease progression remains to be explored. It is possible that these specific phospholipids serve as precursor for NPD1 synthesis or the specific PE di-DHA molecule positively affects autophagy [81]. Thus, a reduction of specific di-DHA molecules in the retina may have to be compensated by much higher DHA blood levels, as uptake from the blood by the RPE may be less efficient.

12.6 Conclusion

Here we discussed the role of PRs in AMD pathogenesis. While it remains unclear what leads to the initial buildup of a lipid wall at the RPE-BrM during the early stages of AMD, the presence of such lipid deposition likely reduces the flow of glucose from the choroidal circulation to PRs [11, 48]. The presence of mitochondrial dysfunction in RPE cells of AMD patients [43] further exacerbates the problem due to the metabolic coupling between the PRs and the RPE [39]. Because PRs have high energy demands, the reprogramming of the intracellular metabolic homeostasis is likely an adaptative response to the nutrient deprivation experienced [37, 38, 82, 83]. However, this adaptive response seems also to contribute to disease progression. The exact sequence of events of this slow progressing early disease stage remains unclear. What is clear is that the metabolic shift in PRs is sufficient to further increase the lipid buildup at the RPE-BrM and contribute to the formation of drusen, GA and CNV [50]. During which stages the complement system plays the most important role remains also unclear. Given the presence of complement components in drusen [10, 23] and the effect of complement inhibition on GA progression seen in clinical trials [27], complement is likely to partake during all disease stages.

A striking finding of our mouse model was the accumulation of lipoproteins at the BrM, the formation of drusen-like deposits driven by changes in cone metabolism, and the reduction in di-DHA containing PE and PC lipids [50]. These findings correlate well with the topographic distribution of rod and cone PRs and their pathologically related subretinal drusenoid deposits and soft drusen, respectively [48, 49]. Furthermore, DHA blood levels [28] or dietary intake [29, 73] has been linked through many epidemiological studies to AMD. The fact that DHA feeding in our mouse model was sufficient to alleviate disease by reducing ApoE accumulation at the BrM and reducing RPE cell stress by improving POS phagocytosis suggests that our model closely mimics important aspects of the human disease [50]. Importantly, DHA feeding did not recover the changes in di-DHA PE and PC lipids in the retina [50]. Since the disease phenotypes seen in our model were independent of the change in the RPE-PR glucose balance [51], the data suggests that gene expression changes that accompany the adaptive metabolic changes to nutrient deprivation are the ones that may contribute to disease [51]. These changes are likely linked to PR lipid metabolism. However, it is unlikely that these changes alone are the sole driving force for disease onset

and progression. A combination of RPE and retinal oxidative stress, in part due to altered PR lipid synthesis caused by a PR adaptive response to nutrient shortage and dysregulated complement, may all equally contribute to disease progression. Further investigations are needed to determine if inhibiting one specific aspect of this complicated chain of events that lead to the human pathology is sufficient to slow disease progression. Our mouse model offers the opportunity to study the chain of events that is initiated by PRs [50].

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