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Retinal phagocytes in age-related macular degeneration

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Abstract

Age-related macular degeneration (AMD) is the leading cause of blindness in industrial countries. Vision loss caused by AMD results from geographic atrophy (dry AMD) and/or choroidal neovascularization (wet AMD). Presently, the etiology and pathogenesis of AMD is not fully understood and there is no effective treatment. Oxidative stress in retinal pigment epithelial (RPE) cells is considered to be one of the major factors contributing to the pathogenesis of AMD. Also retinal glia, as scavengers, are deeply related with diseases and could play a role. Therefore, therapeutic approaches for microglia and Müller glia, as well as RPE, may lead to new strategies for AMD treatment. This review summarizes the pathological findings observed in RPE cells, microglia and Müller glia of AMD murine models.

Keywords

Age-related macular degeneration; retinal pigment epithelium; microglia; Müller glia

Introduction

Age-related macular degeneration (AMD) is the principal cause of blindness in the elderly population of developed countries, including the US. An early clinical sign of AMD is the accumulation of drusen, yellowish deposits in fundus image (Fig. 1A). Vision loss is either due to retinal pigment epithelium (RPE) and photoreceptor cell death, called geographic atrophy (dry AMD) or from the secondary effects of choroidal neovascularization (CNV, wet AMD). Currently, the pathogenesis of AMD is not fully understood, however advanced age, ocular pigmentation, dietary factors, family history, high blood pressure and smoking are well-established risk factors [1–3]. The purpose of this review is to discuss the contribution of RPE, microglia and Müller glia to AMD pathogenesis, especially based on the findings in murine models.

Eye and light hazard

The eye receives light input from the environment and transfers it to the brain, allowing it to be converted into visual information. Light enters the cornea, passes through the pupil and aqueous humor, then lens and vitreous, and finally arrives to the retina at the back of eye

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(Fig. 1B). Light-sensitive cells called photoreceptors, located in the outerlayer of the retina, next to the RPE (Fig. 1C), transfer light energy into electrochemical signals for transmission to the brain. Photoreceptors are classified into two groups, rods and cones. Rods are responsible for night vision and cones are responsible for day and color vision. Light signals are sent from photoreceptors, through the bipolar cells, into the ganglion neurons whose axons form the optic nerve and travel into the thalamic region of brain. Adjacent to the retinal tissue is the RPE, a monolayer of cuboidal cells. RPE cells have apical microvilli, which are associated with the outer segments of photoreceptor cells. The basal membrane of the RPE is in contact with Bruch's membrane, which separates the RPE from the vascular choroid.

Mice are nocturnal and use their noses and whiskers largely, instead of eyes. Some researchers even believe that mice are blind and therefore studying vision in mice is not informative, but the basic anatomical eye structures between human and mouse are same. The main differences between human and mouse eyes is that the mouse has no maculae and a dominant number of rod photoreceptors (97%), while the human macula (around 6 mm in diameter) has predominantly cone photoreceptors, and an especially tiny area of macula called the fovea (0.8 mm) contains only cones. Despite these differences, mouse AMD models have certain pathological, physiological and biochemical features of human AMD such as A2E accumulation, abnormal ERGs, RPE and photoreceptor degeneration, and even neovascularization [4].

Light damage is one of the prominent methods to trigger AMD-like phenotypes in mice. Although light initiates the phototransduction cascade in photoreceptors and relays visual information, at the same time, light can also damage the eye if it is exposed to too much. Experimental work in AMD animal models have shown that light can lead to, or augment, retinal damage to the RPE [5] and photoreceptors [5, 6]. Although most ultraviolet radiation is absorbed by the cornea and lens, a fraction with wavelengths shorter than 400 nm does reach the retina [7]. Ocular light transmission is especially high in young eyes, while the transmission decreases with increasing age. Further, clinical reports indicate that cataract surgery may increase AMD development or progression [8]. Therefore, forming habits to protect the eyes at earlier ages, such as wearing sunglasses, might be more important than we think.

Drusen

Early AMD is characterized by the thickening of Bruch's membrane, lipofuscin accumulation in RPE and drusen formation between the basal RPE and Bruch's membrane. Drusen are generally found between the RPE and Bruch's membrane, but they also form in the subretinal space between RPE and photoreceptors as subretinal drusen or drusenoid deposits [9–12]. Drusen are clinically described as hard or soft. Hard drusen have well defined borders and are relatively common in the elderly population with or without AMD, whereas soft drusen are irregular with fuzzy edges [13]. The presence of many drusen, or drusen of large size, especially soft ones, are significant risk factors for late stage AMD development [14].

The precise mechanisms of drusen formation are still unclear, however the focal deposits include proteins such as vironectin [15], apolipoproteins [16, 17], immune mediated markers [18–20], lipids [21, 22] and trace elements [23, 24]. The presence of inflammation-related molecules in drusen suggests the possible involvement of the immune system in AMD pathogenesis, and a study in age-related changes demonstrates recruitment of leukocytes and activation of the complement cascade in mouse RPE and choroid [25]. Therefore, I will review the pathological roles of retinal phagocytes including RPE, microglia and *Müller* glia in AMD mouse models. Other AMD pathological aspects such as molecular and genetic findings have been previously reviewed [13, 26].

RPE

RPE is a monolayer of largely hexagonal cells located subjacent to the neural retina (Fig 2A). As an epithelium, RPE cells have apical microvilli and a basal surface. The apical microvilli envelop the outer segments of photoreceptors. One RPE cell supports 30–50 photoreceptors [27]. The basal surface of RPE is separated from the vascular choroid by Bruch's membrane.

RPE is essential for the normal function and survival of photoreceptors. It regulates transport of ions, nutrients, and water to and from the blood supply to the subretinal space, and also is one of the components of the outer blood-retinal barrier that blocks nonspecific diffusion and transport from the choroid. RPE apical microvilli extend long processes that wrap around the tips of photoreceptor outer segment (POS) and engulf outer segment shed from photoreceptors during their regenerating process. The most important function of RPE cells is to maintain the retinoid visual cycle by regenerating and moving 11-cis retinal back to photoreceptors. Failure of phagocytosis, renewal of POS and/or visual cycling induces retina degeneration with AMD like phenotypes, as observed in knockout mice such as alphavbeta5 integrin, ATP-binding cassette transporter 4 (ABCA4) and all-trans-retinol dehydrogenase 8 (RDH8) [28–31]. Lack of alphavbeta5 integrin affects RPE phagocytic function [28], and the loss of ABCA4 and RDH8 accumulates all-trans-retinal and N-retinyl-N-retinlyidene-ethanolamine (A2E) lipofuscin, which induces NADPH-oxidase-mediated overproduction of intracellular reactive oxygen species [30–32].

In AMD mouse models such as DICER1 [33], SOD1 [34], Nrf2 [35] and AhR [36, 37] knockouts, or with age, lipofuscin and/or the debris derived from RPE are extruded to the inner collagenous layer of Bruch's membrane, and complement activation and immune complex deposition occurs in RPE, choroid and subretinal space, inducing an infiltration of microglia to aid in the clearance of drusen, and eventually leads to RPE atrophy. Lipofuscin accumulation and oxidative stress in RPE is the major reason for the induction of geographic atrophy. Vascular endothelial growth factor (VEGF), the major angiogenic factor in the pathology of wet AMD, is secreted by the RPE [38, 39]. *In vitro* RPE cells under oxidative stress conditions were reported to secrete VEGF as an autocrine survival factor [40], and VEGF could be one of factors to recruit microglia [41]. RPE degeneration and/or CNV lead to the breakdown of the outer blood retina barrier that RPE maintains. Through these processes, complement activation and immune complex deposition would be accelerated around the RPE atrophy and CNV areas.

Microglia

Microglia are the resident immune cells in central nervous system (CNS), and members of the mononuclear phagocyte system with other macrophages, monocytes and dendritic cells. They have specific differences in density, phenotypes and responsiveness throughout the CNS, including brain, spinal cord and retina. As a member of mononuclear phagocytic cells, microglia survey the environment, and detect subtle cellular damage, then engulf debris and dying cells. However, even in healthy brain, microglia are highly active and dynamic [42, 43], controlling the neural circuit wiring of the nervous system by regulating cell death, pruning and synapse maturation and plasticity [44–46].

Retinal microglia are observed in the outer and inner plexiform (retinal synaptic) layers (Fig. 2C), starting from postnatal day 12 (P12), when synaptogenesis begins to happen in retina. The location and timing of retinal microglia appearance in the plexiform layers suggest that they play a role in synaptic maturation and maintenance as well, like brain microglia. Also, retrograde labeling of retinal ganglion cell (RGC) suggested microglia phagocytosis of dying RGC around P5, the time point when ganglion cell death peaks in the retina [47].

In the aging retina, microglia located in OPL tend to translocate to the subretinal space, and display engulfed melanin or autofluorescent particles (Fig 2D–F). Microglia phagocytosis performance over time is diminished, losing motility, branching complexity and process length with age, although the number of microglia increases slightly [48]. Transcriptome analysis showed increased levels of innate immune and complement genes compared to young mice [49], and isolated microglial cells showed proinflammatory markers [50].

It is unclear how retinal microglia contribute to AMD pathogenesis. However, mice with impaired macrophage recruitment and/or function, such as monocyte chemoattractant protein-1 (Ccl-2), its receptor cognate C-C chemokine receptor-2 (Ccr-2) or CX3CR1 knockouts exhibit AMD phenotypes [51, 52]. AMD mouse models have revealed activated microglia in the subretinal space, with engulfed particles of photoreceptor [52], pigmented and/or autofluorescence [37]. Microglial activation emerges concurrently with, or prior to, the onset of overt retina degeneration and angiogenesis [37, 52–56]. One postulated mechanism of activation is that secreted molecules from stressed RPE recruit and activate microglia, which accelerates severe tissue damage and CNV formation.

Microglia play an important role in tissue maintenance by phagocytosing unhealthy, malfunctional neuron debris and secreting neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF). Thus, maintenance of microglia health in the retina might slow down the onset of age-related degeneration and its progression.

Müllerglia

Müller glial cells are a special type of glia only found in retina, which span the entire neural retina from the inner limiting membrane of vitreous surface to the outer limiting membrane of subretinal space (Fig. 3A). Most importantly, they support all kinds of retinal neurons. Nutrients, waste products, ions, water, and other molecules are transported through Müller

glia from retinal blood vessels to retinal cells [57]. Müller glia form the inner retinal blood barrier by surrounding capillary endothelial cells and the pericytes [58]. Also, Müller glia regulate synaptic activity by uptake of released glutamate and GABA neurotransmitters, and then supply glutamine, the precursor of neurotransmitters, to neurons [59]. The uptake of glutamate by Müller glia contributes to the rapid termination of synaptic action in the inner retina, providing spatial resolution of synaptic activity, and is also considered a protective mechanism from glutamate toxicity [60]. Müller glial cells are especially critical to the viability of cone photoreceptors (Fig. 3B and C). They phagocytize the outer segment discs shed from cone photoreceptors [61], and supply 11-cis-retinol to cone photoreceptors at a 20-fold faster speed than the RPE mediated cycle [62].

In stress conditions, Müller glia produce antioxidants including glutathione [63], lactate [64], alanine [65], α-ketoglutaratet [64], metallothionein [66]and cerulplasmin [67]. However, Müller glia undergo reactive gliosis upon exposure to chronic stresses, injuries, or pathogenic conditions. Gliosis, or glia activation, is protective and helpful to recover homeostasis of neurons, but may be harmful to the retina and contributes to retinal degeneration in chronic situations.

AMD mouse models exhibit Müller glia activation by showing GFAP reactivity, but the involvement of gliosis in the AMD pathogenesis is not clear. On the other hand, activated Müller glia under hypoxic or diabetic conditions secrete pro-angiogenic factors, such as VEGF and basic fibroblast growth factor, having endothelial angiogenic activity [68–70]. Therefore, further study of Müller glia during AMD pathogenesis would be valuable, and Müller glia could be one therapy target that contributes to the maintenance or recovery of healthy retinal function in the future.

Perspective

RPE and Müller glia are essential for normal retina function and the survival of photoreceptors and other retinal neurons by regulating transport of ions, nutrients, and water to and from the blood supply, and constitute outer/inner blood-retinal barriers. Currently, overloaded oxidative stress occurring in RPE is considered to be one of the main causes of AMD phenotypes. However, Müller glia activation is observed in AMD patients [71] and mouse models [37], suggesting contribution to some aspects of AMD pathogenesis, and a possible interplay with microglia migration and activation.

Müller glia activation and the recruited microglia are considered to play protective roles, but in chronic conditions, they can be harmful, accelerate atrophy, and stimulate CNV. RPE is a major target for AMD treatment, but the manipulation of microglia and Müller glia will provide additional benefits.

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List of abbreviations

AMD	Age-related macular degeneration
RPE	retinal pigment epithelium
POS	photoreceptor outer segment
VEGF	Vascular endothelial growth factor
CNS	central nervous system
RGC	retinal ganglion cell

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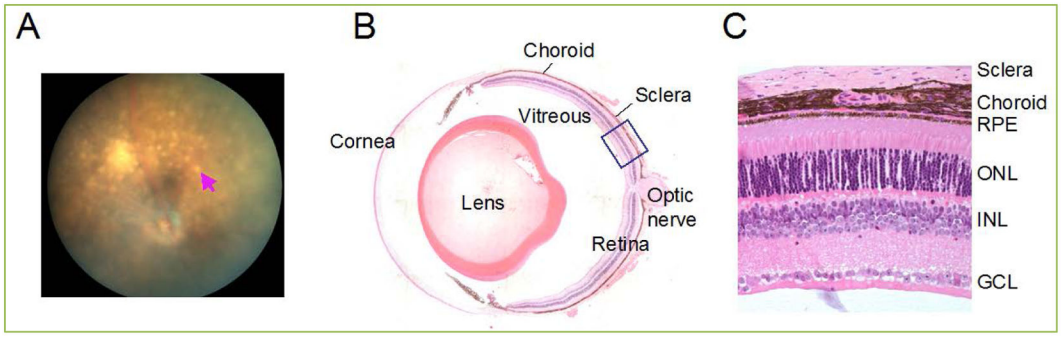

Figure 1. Eye Structure

(A) Fundus image of aryl hydrocarbon receptor (AhR) knockout, one of AMD model mice showing yellowish-white spots (arrow). (B) Cross-section of the adult mouse eye. (C) Retinal histology section zoomed from boxed area in B. Abbreviations: GCL, ganglion cell layer; INL, Inner nuclear layer; OD, optic disc; ONL, outer nuclear layer; RPE, retinal pigment epithelium.

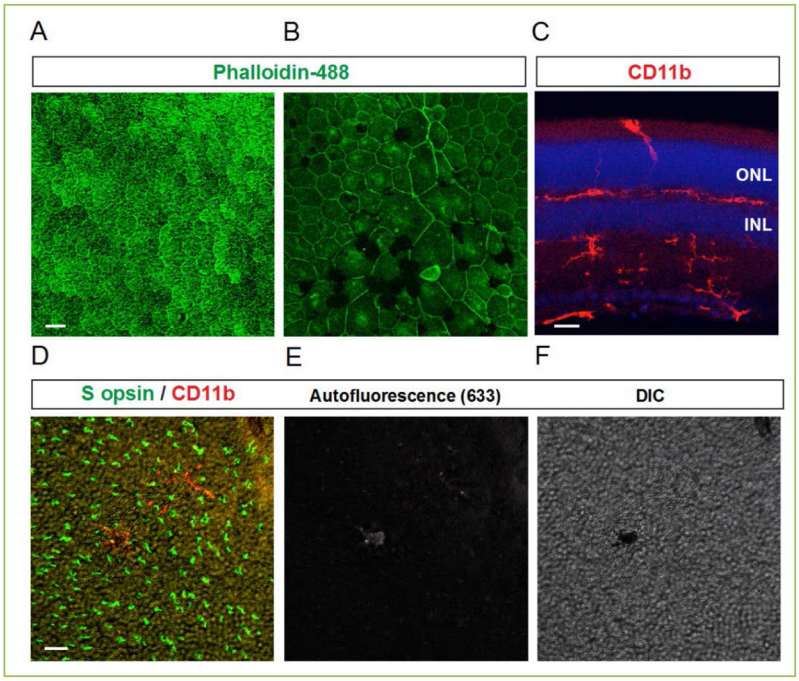

Figure 2. RPE and microglia

(A–B) Horizontal RPE whole mounts of 12-month-old wild type (A) and AhR$^{-/-}$ (B) mice, a model of AMD, stained by phalloidin-488. (C) Vertical image of a 12-month-old wild type retina stained with CD11b microglia marker (red). (D–F) Horizontal retina whole mounts of 12-month-old AhR$^{-/-}$ mouse stained with S opsin and CD11b antibodies showing the presence of autofluorescent microglia in subretinal space. The images of autofluorescence (633 channel) and DIC are provided in E and F, respectively. Abbreviations: INL, inner nuclear layer; ONL, outer nuclear layer. Scale bars: 25 μm in A, B, 20 μm in C–F.

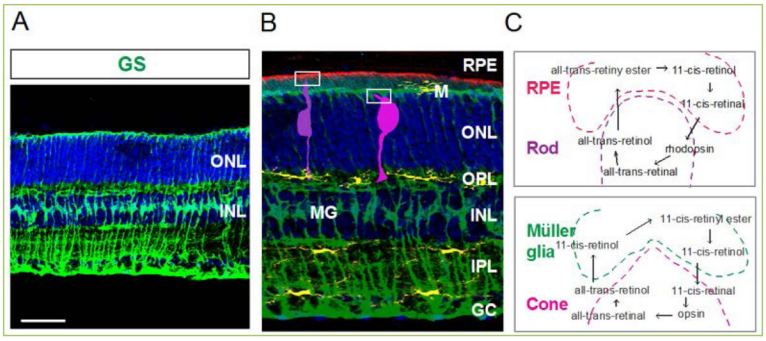

Figure 3. Müller glia

(A) Vertical image of a 6 month-old wild type retina stained with Müller glia marker, glutamine synthetase antibody (green). (B–C) Schematic illustration of microglia (M, yellow) and Müller glia (MG, green) in the vertical view of a murine retina. RPE epical microvilli are descriptive with color of red, and rod and cone photoreceptors are indicated with dark and bright purple colors, respectively (B). Visual cycles between RPE and rods, and between Müller glia and cones (C). GC, ganglion cell layer, INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OPL, outer plexiform layer; RPE, retinal pigment epithelium. Scale bar: 50 μm in A.