Editorial

Cholesterol homeostasis, macrophage malfunction and age-related macular degeneration

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Introduction

Age related macular degeneration (AMD) is the leading cause of blindness in the elderly in developed countries (1,2). Due to the growing aging population and increased life expectancy, the number of AMD cases is expected to be doubled by 2020 (3). The early stage of AMD is characterized by the deposition of extracellular materials beneath (basal laminar and basal linear deposition and drusen) or above (pseudo-drusen) the retinal pigment epithelium (RPE). The disease can advance into two late stages, "dry" and "wet". Dry AMD refers to the geographic atrophy of the macula; whereas wet AMD is the growth of abnormal blood vessels into the macula, also known as neovascular AMD (nAMD). Although nAMD is treated by intravitreal injection of vascular endothelial growth factor inhibitors (e.g., anti-VEGF antibodies), the treatment is not curative, and patients eventually lose their central vision due to the development of geographic atrophy or macular fibrosis (4). Dry AMD is more common than the wet form and there is currently no treatment for it. AMD remains to be a major medical and socioeconomic challenge worldwide. Therefore, there is an urgent need for better therapies or prevention, and further understanding the underlying mechanisms of the disease is essential to achieve this.

Macrophage dysfunction in AMD

Macrophage dysfunction plays an important role in the pathogenesis of AMD (5,6). Macrophages may contribute to the development of AMD at multiple levels. When the macula suffers from oxidative insults, microglia and macrophages may migrate to the subretinal space (7), the junction between photoreceptor and RPE cells (*Figure 1*), with the intention to clean debris and maintain homeostasis, a phenomenon known as para-inflammation (8,9). Choroidal macrophages may also infiltrate into the sub-RPE space (2,10) (*Figure 1*). Once they phagocytize debris, they are removed from the subretinal space and sub-RPE space, and impaired removal of these cells causes subretinal inflammation and photoreceptor degeneration (6). If subretinal phagocytes are unable to remove debris, particularly when the RPE function is compromised and waste materials cannot be digested or transported by RPE to the choroid, subretinal drusenoid deposits (also known as pseudo-drusen) may occur.

Choroidal macrophages are important scavenger cells responsible for the removal of waste materials and debris from the Bruch's membrane and choroidal parenchyma (*Figure 1*). Malfunction of choroidal macrophages may lead to the development of basal laminar deposits and drusen. Drusen consists of cholesterol-rich lipids and various oxidized proteins, which can induce inflammasome activation (11), and complement activation (12) leading to the death of RPE and photoreceptors, cardinal features of dry AMD.

In wet AMD, the development of macular blood vessels such as choroidal neovascular membrane (CNV) (*Figure 1*)

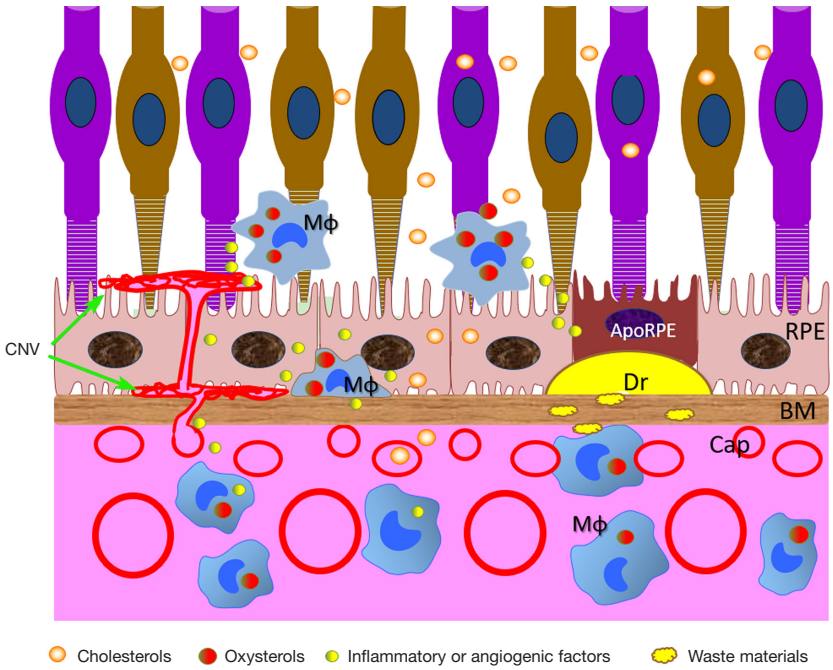

Figure 1 Cholesterol homeostasis and macrophage malfunction at the retina-choroidal interface in age-related macular degeneration. Photoreceptors acquire cholesterols and fatty acids from choroidal circulation or synthesize internally using acetyl-CoA to maintain the high-rate of photoreceptor outer segment (POS) turnover. The POS is phagocytized and digested by RPE cells. Retinal waste materials are dumped to choroid and then removed by choroidal macrophages. During aging, RPE function declines leading to the accumulation of undigested POS at the subretinal space or within RPE cells (in the form of lipofuscin). Macrophages and microglia migrate to subretinal space to remove undigested lipid-rich POS and damaged RPE debris. Macrophages may also migrate to sub-RPE space to remove debris. In AMD, macrophages are unable to maintain intracellular cholesterol/fatty acid homeostasis resulting in the accumulation of oxidized fatty acid (e.g., oxysterol). Intracellular oxysterol may activate various signalling pathways leading to macrophage malfunction. These include (I) reduced phagocytosis, which may be related to drusen formation; (II) uncontrolled inflammatory cytokine production, which may lead to RPE and photoreceptor death and the development of dry AMD; (III) the production of pro-angiogenic growth factors, which may induce wet AMD. ApoRPE, apoptotic RPE; BM, Bruch's membrane; Cap, choriocapillaris; CNV, choroidal neovascularization; Dr, drusen; MΦ, macrophages; RPE, retinal pigment epithelium.

is driven by abnormal levels of VEGF, which is why intravitreal injection of VEGF inhibitors is effective in treating the disease. Although multiple types of cells in the macula may produce VEGF in wet AMD, macrophage-driven inflammation is known to play a pivotal role (13). Infiltrating macrophages have been detected in human AMD (14). Myeloid cells from AMD patients express higher levels of phosphorylated STAT3 (15) and produce higher levels of VEGF, IL6 and are more proangiogenic when cultured in vitro (16). Macrophages can directly or indirectly participate in retinal angiogenesis in wet AMD.

Macrophages, as an important type of innate immune cells, are supposed to clean debris and promote tissue repair when macula suffers from oxidative damage. Why macrophages become detrimental in AMD still remains elusive.

Lipid metabolism and AMD

Fatty acids such as cholesterol and triglycerides are important fuel source of the retina. In the outer layers of the retina, lipid-rich photoreceptor outer segments are regenerated daily to recycle the light-sensitive visual pigments (e.g., retinal). Photoreceptors maintain the metabolic demands by taking up a large amount of fatty acids through free tatty acid receptor 1 (Ffar1) and very-low-density lipoprotein receptor

(VLDLR) (17). The fatty acids are acquired exogenously from choroidal circulation, although they can also be generated endogenously through the glycolysis pathway i.e., acetyl coenzyme A (acetyl-CoA) (18). Dysregulated lipid metabolism is known to play an important role in the development of AMD although the underlying mechanism needs further investigation.

Impaired glucose and fatty acid uptake in photoreceptors results in a reduction in the levels of the Krebs cycle intermediate α-ketoglutarate. Low levels of α-ketoglutarate stabilize hypoxia-induced factor 1α (HIF1α) and induces retinal angiogenesis including wet AMD (17). Although evidence supporting glucose and fatty acid deficiency in AMD barely exists, compelling evidence suggests that dysregulated cholesterol and lipid homeostasis plays a pivotal role in AMD (18). The polymorphisms of genes related to lipid/lipoprotein metabolism have been associated with AMD (18). Higher circulating levels of high-density lipoprotein-cholesterol (HDL-C) are associated with increased risk for AMD (19). HDL can remove cholesterol from cells and is considered to be protective [compared with low density lipoprotein (LDL)]. It is possible that the structure of cholesterol within the HDL in AMD patients may differ from that in healthy controls. Indeed, a recent study by Lin *et al.* has shown that plasma levels of oxidized cholesterols, the oxysterols, in particular the 24-hydroxycholesterol can discriminate physiological aging from AMD (20). The study highlights the role of oxidized cholesterols in the development of AMD.

Lipid metabolism dysregulation and macrophage malfunction in AMD

Although the pathogenic roles of macrophage malfunction and cholesterol/lipid metabolism dysregulation in AMD have been recognized for over a decade, little is known on how the two phenomena are linked. Macrophages are specialized in metabolizing lipid and this function is needed for them to eliminate cholesterol that they acquire through the phagocytosis of dying cells. On the other hand, macrophage activation and polarization are regulated by the metabolic pathways. For example, the classically activated M1 macrophages are fuelled by the glycolysis pathway, whereas the alternatively activated M2 macrophages relay on fatty acid oxidative phosphorylation (21). The study by Lin and colleagues (20) suggests that macrophage malfunction in AMD may be related to cholesterol metabolism dysregulation. First, the authors showed that mitochondrial oxidative phosphorylation is considerably impaired in aged macrophages. Using transcriptomic approaches, the authors discovered global impairments in cholesterol and lipid homeostasis, including cholesterol/lipid biosynthesis, elimination, transport and uptake (20). Importantly, they found that the intracellular levels of oxidized cholesterol including 4β-hydroxycholesterol, 7-ketocholesterol and cholestane-3β, 5α, 6β-triol were higher in aged macrophages, particularly after treatment with oxidized LDL (20).

This observation is important as accumulation of intracellular cholesterol can lead to activation of several transcription factors, including the liver X receptor α and β (LXRα and LXRβ), the retinoid X receptor (RXR) and member of the peroxisome proliferator-activated receptor (PPAR) family including PPARα and PPARγ (22). Normally, the LXR and RXR would form heterodimers that can upregulate the expression of the ATP-binding cassette subfamily A member 1 (ABCA1) and ABCG1 (22), which can then regulate the efflux of free cholesterol in order to maintain cellular cholesterol homeostasis. This regulatory mechanism appears to be impaired in aged macrophages, particularly in AMD. The expression of ABCA1 and cholesterol efflux is known to be reduced in aged macrophages (23), and ABCA1 polymorphisms are associated with advanced AMD (24). Statins, which inhibits the 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase and reduces cholesterol production from acetyl-CoA, can reduce some high-risk features of AMD (25). Furthermore, activation of the PPARs is also shown to be involved in the development of AMD (26). Taken together, these results suggest that accumulation of intracellular oxysterols in macrophages may confer their pathogenic phenotype leading to the development of AMD.

In the context of AMD, impaired macrophage scavenger function may be related to the development of drusen and pseudo-drusen, whereas uncontrolled macrophage activation towards pro-inflammatory or pro-angiogenic phenotype may lead to the development of dry or wet AMD (*Figure 1*). Further studies are required to understand why and how cholesterol metabolism is dysregulated and which signalling pathways and functions of macrophage are affected by intracellular oxysterols in AMD.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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