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Potential of autophagy in subretinal fibrosis in neovascular age-related macular degeneration

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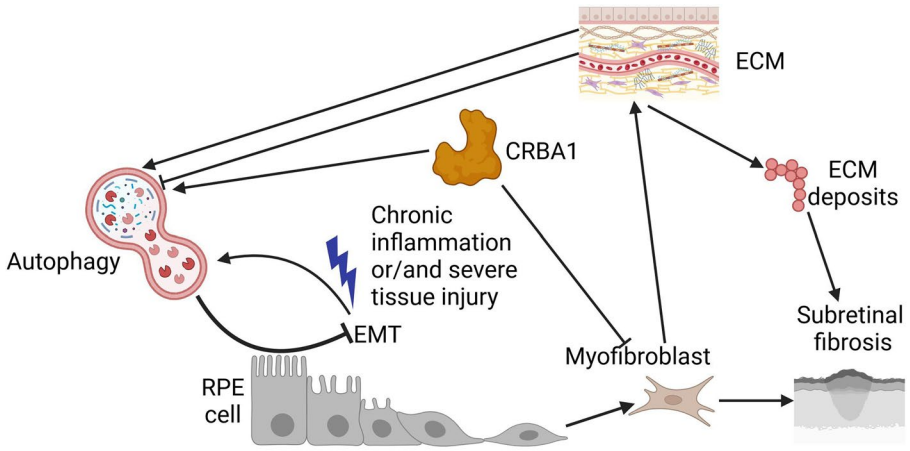
Abstract

Age-related macular degeneration (AMD) is an eye disease that can lead to legal blindness and vision loss. In its advanced stages, it is classified into dry and neovascular AMD. In neovascular AMD, the formation of new blood vessels disrupts the structure of the retina and induces an inflammatory response. Treatment for neovascular AMD involves antibodies and fusion proteins targeting vascular endothelial growth factor A (VEGFA) and its receptors to inhibit neovascularization and slow vision loss. However, a fraction of patients with neovascular AMD do not respond to therapy. Many of these patients exhibit a subretinal fibrotic scar. Thus, retinal fibrosis may contribute to resistance against anti-VEGFA therapy and the cause of irreversible vision loss in neovascular AMD patients. Retinal pigment epithelium cells, choroidal fibroblasts, and retinal glial cells are crucial in the development of the fibrotic scar as they can undergo a mesenchymal transition mediated by transforming growth factor beta and other molecules, leading to their transdifferentiation into myofibroblasts, which are key players in subretinal fibrosis. Autophagy, a process that removes cellular debris and contributes to the pathogenesis of AMD, regardless of its type, may be stimulated by epithelial–mesenchymal transition and later inhibited. The mesenchymal transition of retinal cells and the dysfunction of the extracellular matrix—the two main aspects of fibrotic scar formation—are associated with impaired autophagy. Nonetheless, the causal relationship between autophagy and subretinal fibrosis remains unknown. This narrative/perspective review presents information on neovascular AMD, subretinal fibrosis, and autophagy, arguing that impaired autophagy may be significant for fibrosis-related resistance to anti-VEGFA therapy in neovascular AMD.

Keywords: Neovascular age-related macular degeneration, Subretinal fibrosis, Autophagy, Epithelial–mesenchymal transition, Endothelial–mesenchymal transition, Transforming growth factor beta 2, Extracellular matrix deposits

Graphical Abstract



Introduction

Age-related macular degeneration is an eye disease whose incidence increases significantly with age and that poses an emerging challenge for the healthcare system owing to the aging population. It impacts mainly the macula, a specialized region of the retina that contains the fovea and is responsible for central, color, and high-resolution vision, but AMD signs can also be observed in the peripheral part of the retina. AMD can lead to severe vision impairment and loss of sight. The highest reported prevalence of AMD is in high-income countries, but this may reflect more accurate disease screening and a longer life expectancy in these regions. Dry AMD, the most common form of the disease, is currently untreatable. Typically, AMD begins as dry AMD but can progress to neovascular AMD, which advances more rapidly, making vision loss more likely compared with the dry form.

The intermediate stage of AMD is characterized by pigmentation irregularities in the retinal pigment epithelium (RPE). Neovascular AMD always follows the early stages of AMD, which are marked by the formation of drusen, extracellular deposits made up of lipids and proteins [1]. In its advanced stage, AMD occurs in two clinically distinguishable forms: dry (nonexudative, atrophic) and neovascular (exudative, neovascular) AMD (Fig. 1). Dry AMD is the initial form of the disease that may progress to neovascular AMD, accounting for 15–20% of all cases of advanced AMD [2]. The late form of dry AMD, known as geographic atrophy (GA), is linked to the death of photoreceptors and the atrophy of supporting retinal pigment epithelium (RPE) cells, as well as the choriocapillaris, which may result in vision loss [3]. Mixed type 1 and 2 MNV represents OCT findings of both type 1 and type 2. Also, MNV3 may be involved in mixed AMD. However, clinically, AMD is always classified as either dry (ICD code 35.30) or neovascular AMD (ICD code 35.31).

Despite many preclinical studies and numerous clinical trials, advanced dry AMD remains untreatable. However, recent results from the FILLY, OAKS, and DERBY clinical trials with pegcetacoplan have shown promise, and the Food and Drug

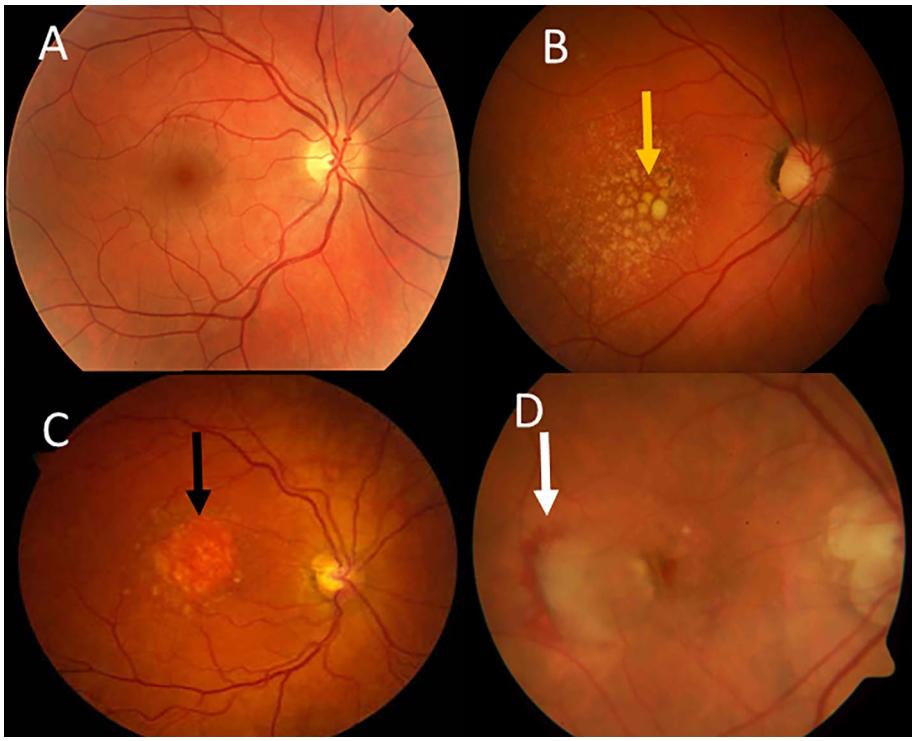


Fig. 1 Age-related macular degeneration (AMD): fundus images of a healthy macula (**A**), the macula in intermediate dry AMD (**B**), late dry AMD characterized by geographic atrophy (**C**), and neovascular AMD (**D**). The yellow arrow indicates drusen, the black arrow points to retinal atrophy, and the white arrow designates hemorrhages. Pictures were extracted from the Kuopio University Hospital Imaging Database by Kai Kaarniranta

Administration (FDA) has approved this drug. It is important to note that it only delays the development of GA compared with untreated controls [4]. In contrast to the FDA, the European Medical Agency (EMA) did not approve GA treatment with pegcetacoplan owing to limited effects on visual acuity.

Despite the established diagnostic criteria and procedures, along with the clear clinical picture of AMD, many patients still miss out on effective treatment. The primary reason for this is an incomplete understanding of AMD pathogenesis mechanisms. This is further emphasized by the insufficient progress in experimental studies on the disease's molecular basis, hindered by restricted access to human target material and the limited value of animal models for human AMD [5]. As a result, there are no AMD-specific preventive recommendations or therapies for GA in Europe, and options for preventing the formation of new vessels in the choroid in neovascular AMD are limited [6]. However, the introduction of medications targeting vascular endothelial growth factor A (VEGFA) and its receptor marked a significant advancement in treating age-related macular degeneration (AMD), leading to a considerable delay in vision loss for patients with neovascular AMD. In certain cases of neovascular AMD, anti-VEGFA treatment proves ineffective, and the reasons for this remain largely unknown [7]. In patients experiencing a recurrence of neovascularization after initial inhibition, alternative pathways to VEGFA may be activated [8]. Furthermore, patients with neovascular AMD constitute a diverse array of anatomical, morphological, and

genetic cases, which may influence the effectiveness of anti-VEGFA treatment [9]. Additionally, the formation of new blood vessels may be associated with leakage and hemorrhage, resulting in an inflammatory response and the engagement of the outer retina in fibrosis [10]. Subretinal fibrosis was noted following anti-VEGFA therapy in patients with myopathic choroidal neovascularization [11, 12]. Therefore, fibrosis may hinder the effectiveness of anti-VEGFA therapy. However, the MARINA study demonstrated that ranibizumab, an anti-VEGFA agent, reduced or delayed the progression of subretinal fibrosis in patients with neovascular AMD [13]. Currently, there is no evidence that anti-VEGFA treatment can directly induce fibrosis, which may develop even with such treatment. The progression of neovascular AMD shares some features in common with abnormal wound healing, often leading to fibrosis, with fibrotic scars being the endpoint of untreated exudative AMD [14].

Fibrosis can be a significant cause of morbidity and mortality in a wide range of disorders [15]. It might contribute to a substantial disease burden, as the prevalence of fibrosis-related disorders is estimated at 5000 per 100,000 person-years [16]. Fibrosis can affect various organs, including the lungs, liver, intestines, kidneys, heart, skin, and oral mucosa [17–23]. Regardless of the cause of organ damage, a common feature of fibrosis is the activation of fibroblasts and the excessive deposition of extracellular matrix (ECM), including collagen and fibronectin [24].

Autophagy, the process of removing and recycling damaged or unneeded cellular components, plays a role in the pathogenesis of AMD [25, 26]. It may play a role in drusen biogenesis, the senescence-associated secretory phenotype, inflammation, epithelial–mesenchymal transition, and other significant effects in the pathogenesis of AMD. Additionally, autophagy has been reported to be impaired in fibrosis across various tissues and disorders, including pulmonary, neural, cardiac, hepatic, and renal diseases; however, no experimental data demonstrate the involvement of autophagy in macular fibrosis related to neovascular AMD [27]. Furthermore, autophagy can present both beneficial and harmful effects in various processes. As a result, it cannot easily be classified into “friend/foe" categories, necessitating an understanding of the cellular context for its specific actions.

Generally, fibrosis can occur at any age, but it is more common in the elderly. In idiopathic pulmonary fibrosis (IPF), aging is the most significant risk factor [28]. In various other interstitial diseases, the fibrotic response intensifies and deteriorates with age [29]. Thus, fibrosis may be viewed not only as a facet of pathology but also as a component of biological aging, as reflected in the concept of “fibroaging” [30].

In this narrative perspective review, we present essential information on neovascular AMD and its treatment, discuss the clinical features and risk factors associated with macular fibrosis secondary to neovascular AMD, and consider the potential role of autophagy in AMD-related fibrosis.

Neovascular AMD and fibrosis

While some cases of neovascular AMD are often initially diagnosed as dry AMD, the precise relationship between these two types of AMD remains unclear, and in their advanced stages, they may be regarded as distinct diseases [31]. We and others

have demonstrated that neovascular AMD may be linked to increased mortality [32]. Recently, it was suggested that persistent central vitreomacular adhesion may be common in both forms of AMD [33].

AMD is a complex disease characterized by the interplay of genetic, epigenetic, environmental, and lifestyle factors in its pathogenesis. Similar to general AMD, the risk factors for neovascular AMD can be categorized into documented and putative. Advanced age is, by definition, the most significant risk factor for AMD; however, there is no consensus on the age threshold for AMD, as the range of 50–65 years is frequently cited, and the consensus from the Neovascular Age-Related Macular Degeneration Nomenclature Study Group states "beyond 50 years" [34]. Thus, chronological aging may initially indicate the actual risk factors tied to aging, while biological aging more accurately represents aging as a risk consideration. However, biological aging cannot be viewed as an independent risk factor, as it is influenced by a person's genetic makeup, environmental factors, and lifestyle choices. Clearly, chronological aging plays a role in AMD pathogenesis, but it should be evaluated alongside other factors to determine its potential as a risk factor. In addition to aging, smoking is consistently reported as the most significant AMD risk factor [35]. Certain variants of the complement factor H (*CFH*), apolipoprotein (*APOE*), age-related maculopathy susceptibility 2 (*ARMS2*), and HtrA serine peptidase 1 (*HTRA1*) genes are the strongest genetic risk factors for AMD [36–38].

Neovascular AMD is marked by the disruption of Bruch's membrane and the development of macular neovascularization (MNV, also known as choroidal neovascularization, CNV) membranes, which are essential for the formation of new blood vessels growing beneath the macula. These vessels extend from the choriocapillaris (CC) through Bruch's membrane into the subretinal space. A reduction in blood supply due to stenosis of the large vessels in the CC can lead to the loss of choroidal vasculature, which, alongside defects in Bruch's membrane, may trigger neovascular AMD [39]. Unlike GA, the RPE remains essential in neovascular AMD, and its cells produce angiogenic factors, including VEGFA, which is necessary for synthesizing new blood vessels from CC [40].

Three subtypes of MNV and, consequently, neovascular AMD—types 1 to 3—can be distinguished on the basis of the localization of the neovascularization's origin [34]. These are type 1: sub-RPE occult MNV membrane (MNV1); type 2: classic subretinal membrane above the RPE (MNV2); and type 3: retinal angiomatous proliferation (MNV3) [34]. The HARBOR study showed that MNV2 lesions were more frequently associated with fibrosis than MNV1 lesions, which resulted from the more extensive neovascularization observed with MNV2 [41]. However, in each MNV subtype, newly formed blood vessels are fragile rather than hermetic and are predisposed to leakage and hemorrhage beneath the RPE or photoreceptors, which can result in severe vision impairment or loss [42]. These devastating effects cause an inflammatory response that leads to the release of stromal and immune cells, prompting the transition of the neovascular endothelial cluster into a fibrovascular membrane [42]. Fibrosis may be beneficial in inhibiting leakage and exudation. However, excessive fibrosis can result

in a fibrotic scar, which is a primary cause of vision loss in neovascular AMD. The development of submacular fibrosis is linked to lesions in the RPE and photoreceptors, leading to irreversible vision impairments, even with anti-VEGFA treatment [43, 44].

The clinical definition of retinal fibrosis, as provided by the Neovascular Age-Related Macular Degeneration Nomenclature Study Group, describes it as the accumulation of tissue in any layers of the retina, including the subretinal space, RPE monolayer, or sub-RPE space [34] (Fig. 2). However, this is a rather general description of fibrosis, and a more specific and practical depiction defines macular fibrosis as a distinctly marked, elevated structure of white-yellowish material within or under the retina that does not show signs of dehemoglobinized blood or hard exudates upon fundus examination [45, 46]. The time course of macular fibrosis can be categorized into three stages: minimal, prominent, and hyperreflective subretinal fibrosis [47].

No specific independent macular fibrotic risk factors have been identified thus far, apart from those associated with AMD. However, some studies report an increased occurrence of macular fibrosis correlated with various parameters of neovascular AMD and general ophthalmologic characteristics. For instance, a large-cohort, multicenter study involving neovascular AMD patients over 10 years found that the independent factors associated with fibrosis included larger variations in central subfield thickness, submacular hemorrhages, and worse baseline visual acuity [48]. Moreover, type 2 MNV was significantly linked to mixed and subretinal fibrosis. Blocked fluorescence in fluorescein angiography indicates extensive basal damage, increased retinal thickness, foveal subretinal fluid, and subretinal hyperreflective material (SHRM) located beneath the center of the fovea at baseline [49, 50]. While the exact specification of SHRM is unclear, it is viewed as a diagnostic marker for macular fibrosis [51].

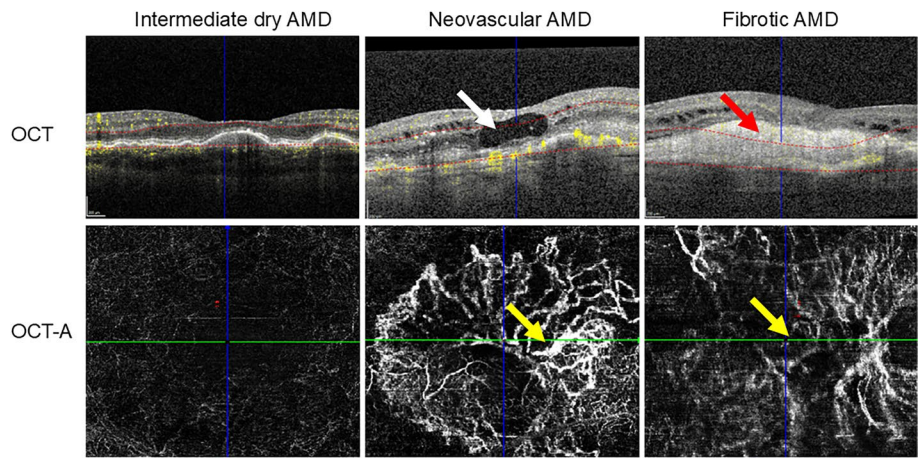


Fig. 2 Optical coherence tomography (OCT) and OCT-angiography (OCTA) images from various phenotypes of age-related macular degeneration (AMD). The white arrow indicates intraretinal fluid, the red arrow points to fibrosis, and the yellow arrows highlight macular neovascularization, which is no longer as active in fibrotic wet AMD. Pictures were extracted from the Kuopio University Hospital Imaging Database by Kai Kaarniranta

Molecular mechanisms of submacular fibrosis

The exact mechanism behind the formation of submacular fibrosis scars remains unknown. It is widely accepted that this process results from prolonged tissue damage and involves several sequential stages, including cellular death and inflammation, cell proliferation and tissue replacement, degradation of the extracellular matrix (ECM), and tissue remodeling [42]. The blood–retina barrier (BRB) regulates the movement of molecules and immune cells from systemic circulation to retinal compartments. The outer BRB comprises retinal pigment epithelium (RPE) cells, Bruch's membrane, and fenestrated endothelium [52]. However, the connections between choroidal endothelial cells can weaken with aging, resulting in increased permeability and the extravasation of macromolecules from choroidal capillaries [53]. The increased permeability may also lead to fluid accumulation and induce low-level inflammation in the surrounding tissue microenvironment because of infiltrating immune cells [54]. Fibrosis in neovascular AMD can result from hypoxia-driven angiogenesis, leading to fibrovascular scarring [55].

Disruption of Bruch's membrane is a critical event in the formation of MNV, as it allows choroidal endothelial cells to proliferate and penetrate, forming new neovasculature. These newly formed vessels are fragile and not hermetic, which supports further tissue damage and leads to the release of inflammatory molecules. A subsequent interplay between various cell types, including myofibroblasts and immune cells, results in excessive ECM deposition and tissue remodeling, leading to fibrotic healing [55]. Macular fibrosis may develop in neovascular AMD owing to vascular damage during the angiofibrotic switch process [56].

The buildup of specialized myofibroblasts is a key factor in the development of subretinal fibrosis, resulting in increased ECM deposition, tissue contraction, and compromised functions [57]. However, myofibroblasts are absent in the normal macula, and their precursors in neovascular AMD have not been clearly identified since the original markers are lost during transdifferentiation [10]. Retinal pigment epithelium cells are the main candidates for this role in subretinal fibrosis related to neovascular AMD [58]. Under normal conditions, RPE cells do not proliferate owing to spatial restrictions mediated by cadherins [59]. However, under certain circumstances, RPE cells may lose their epithelial phenotype and undergo a mesenchymal transition, which contributes to ECM deposition and MNV progression [60]. Therefore, RPE cells and their epithelial–mesenchymal transition (EMT) may be crucial for subretinal fibrosis [42].

EMT can be viewed as a continuum in which cells display epithelial (E), intermediate (EM), and mesenchymal (M) phenotypes. As cells transition, they sequentially lose apicobasal polarity and cell–cell adhesions while gaining front–back polarity and enhanced cell–matrix interactions. EMT regulators include transcription factors such as SNAI1/2 (zinc finger protein SNAI1/2), ZEB1/2 (zinc finger E-box-binding homeobox 1/2), TWIST1 (twist-related protein 1), GRHL2 (grainyhead-like protein 2 homolog), OVOL1/2 (putative transcription factor Ovo-like 1), and PRRX1 (paired mesoderm homeobox protein 1), as well as miRNAs and other epigenetic control factors at the promoters of epithelial and mesenchymal genes [61].

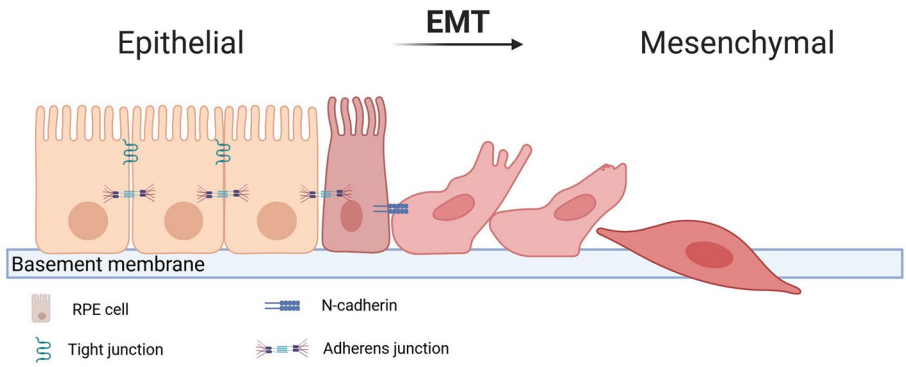


Fig. 3 Epithelial–mesenchymal transition (EMT) in retinal pigment epithelial (RPE) cells. During EMT, the cuboidal, polarized, tightly adjacent, and nonmotile RPE cells transdifferentiate into spindle-shaped mesenchymal cells that acquire migratory and contractile properties and lose mutual contact. Created with BioRender.com

EMT in RPE cells is linked to the loss of apicobasal polarity, transformation to fibroblastic morphology, and the development of migratory mesenchymal characteristics (Fig. 3) [62].

Another source of myofibroblasts can be endothelial cells that undergo endothelial–mesenchymal transition (EndT) [63]. Several other cell types—such as fibrocytes, pericytes, and myeloid cells, including macrophages—can be involved in fibrosis associated with neovascular AMD [64].

Inflammation plays a crucial role in the development of neovascular AMD [39]. Myeloid cells, including blood-derived macrophages and resident retinal microglia, are the primary components linking inflammation to neovascular AMD, as they stimulate the neuroinflammatory cycle, resulting in tissue damage, and promote the proliferation and differentiation of stromal cells through the production of pro-inflammatory and pro-fibrotic mediators [65]. Microglia can express proinflammatory molecules, such as interleukin-1 beta (IL1B), interleukin-6 (IL6), and tumor necrosis factor (TNF), as observed in Alzheimer's disease [66]. If such a release occurs at the site of retinal damage, it can worsen fibrosis.

Growth factors facilitate communication between molecules involved in the fibrosis process [67]. The primary mediator is transforming growth factor beta-2 proprotein (TGFB2), which plays a role in the mesenchymal transformation of macrophages, epithelial, endothelial, and other cell types [68]. TGFB2 was found in MNV and retinal fibrosis scars, and its expression was positively correlated with the severity of the scars (reviewed in Ref. [42]). In RPE cells, TGFB2 induces EMT primarily through the mothers against the decapentaplegic homolog 3 (SMAD3) signaling pathway [69]. Another important growth factor for fibrosis is the connective tissue growth factor (CTGF), which regulates the mechanisms that lead from wound healing to fibrosis [70]. Inhibition of CTGF led to reduced fibrosis in the mouse laser-induced MNV model [71]. The same model demonstrated that fibroblast growth factor 2 (FGF2), a member of the TGF family, reduces subretinal fibrosis [72]. Platelet-derived growth factor (PDGF) is a key player in idiopathic pulmonary fibrosis and may also play an important role in fibrosis in other areas of the human body [73]. Blocking the PDGF receptor beta (PDGFRB) in a laser-induced MNV mouse model led to a reduction in MNV and decreased subretinal

fibrosis [74]. However, study of the PDGFB inhibitor pegpleranib was discontinued in phase III owing to limited efficacy [75].

Leaky vessels are linked to the extravasation of fibrinogen from the vascular system [76]. Thus, fibrosis can arise from abnormal wound healing, which in its early phase includes the formation of a provisional ECM containing fibrin, fibrinogen, laminin, and fibronectin [77]. Interestingly, thrombin, an enzyme that converts fibrinogen into fibrin, has been shown to reduce transepithelial resistance in RPE cells, generate complement C3/C5 cleavage products, and increase the expression of connective tissue growth factor and VEGF [78]. Anticoagulants' interference with thrombin action may also delay the onset of neovascular AMD and help patients maintain anti-VEGF treatment for a longer duration [78, 79]. Additionally, studies have shown that the direct thrombin inhibitor dabigatran can reduce pulmonary fibrosis [80]. Excessive buildup of collagen and other extracellular matrix (ECM) components occurs in the later stages of fibrosis. Fibroblasts reside in this provisional ECM and multiply in response to signals released by leukocytes that migrate into the wound and are retained by the ECM structure [77]. Extracellular matrix components, including fibronectin, collagen, and laminin, are essential elements of MNV membranes, and their accumulation contributes to retinal fibrosis [81]. Several other ECM components were identified as playing a role in MNV, including periostin, a secreted cell-adhesion protein that functions as a ligand for alpha-V/beta-3 and alpha-V/beta-5 integrins. Thus, it supports epithelial cell adhesion and migration and is considered an anti-fibrotic target in neovascular AMD therapy [82, 83].

The harmful role of fibrosis in neovascular AMD suggests that targeting it could be beneficial for patients, yet, at present, there is no approved anti-fibrotic treatment for individuals with neovascular AMD [42]. Clinicaltrials.gov lists four clinical trials directly related to fibrosis in neovascular AMD: one completed, one terminated, and two of unknown status (https://clinicaltrials.gov/search?cond=AMD%20-%20Age-related%20Macular%20Degeneration&term=Fibrosis, accessed 10 January 2025). Some ongoing trials are evaluating anti-VEGF treatment combined with other drugs, with fibrosis as one of the outcomes.

In summary, although the exact mechanism of retinal fibrosis is not fully understood, the mesenchymal transformation of RPE cells, choroidal fibroblasts, and retinal glial cells into myofibroblasts through EMT or EndMT may be a crucial event in this process (Fig. 4). Mechanistically, retinal EMT and EndMT in AMD may be driven by various signaling pathways, some of which are presented in Fig. 4, while more details can be found in other reviews, e.g., [63, 84, 85]. These signaling pathways are mediated by numerous factors, including cytokines, growth factors, and ECM components, with TGFB2 serving as the master regulatory protein. Retinal fibrosis may be induced by chronic low-level inflammation and/or retinal injury, which are typical of advanced neovascular AMD. In the subsequent sections, we present arguments that impairment of autophagy may worsen the formation of fibrotic lesions.

While we focus on AMD, the issue of shared and distinct mechanisms in subretinal diseases within AMD and other retinal disorders remains. Subretinal fibrosis can be triggered by several factors, including inflammation, cell proliferation, ECM expansion, vascular leakage, neovascularization, and hemorrhage, ultimately leading to fibrovascular tissue formation and significant vision loss [86]. During fibrosis, the retina

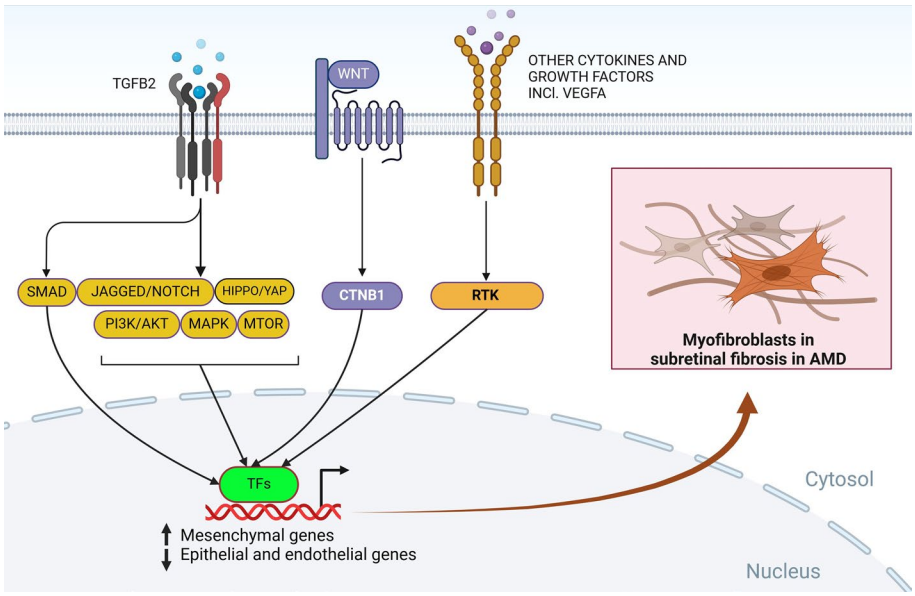


Fig. 4 Signaling pathways and mechanistic drivers involved in mesenchymal–epithelial transition (EMT) and endothelial–mesenchymal transition (EndMT) in neovascular age-related macular degeneration (AMD). The action of numerous extracellular cytokines and receptor tyrosine kinase (RTK) activates EMT of retinal pigment epithelial cells and EndMT of choroidal endothelial cells. TGFB2 is the master regulator, activating the canonical SMAD and noncanonical signaling pathways. The WNT/CTNB1 (catenin beta-1) pathway also plays a key role in both EMT and EndMT, whose activation results in the upregulation of several transcription factors (TFs) of mesenchymal genes and the downregulation of TFs of epithelial and endothelial genes. This ultimately leads to transdifferentiation of epithelial and endothelial cells to myofibroblast and excessive extracellular matrix deposition, occurring in subretinal fibrotic lesions in AMD. JAGGED/NOTCH, protein Jagged1/2/neurogenic locus notch homolog protein 1; YAP, transcription activator yes 1; PI3K, phosphatidylinositol 3-kinase; AKT, AKT serine/threonine kinase 1; MAPK, mitogen-activated protein kinase; MTOR, mechanistic/mammalian target of rapamycin. Created with BioRender.com

undergoes wound-healing processes, which include the removal of injured tissue, cell proliferation and migration, neovascularization, cytokine-mediated interactions, and remodeling of the underlying ECM [87].

Epiretinal membranes (ERMs) are fibrocellular proliferations on the internal limiting membrane of the macula, likely resulting from glial cell proliferation [88, 89]. ERMs can be either idiopathic or associated with posterior vitreous detachment, DR, PDR, proliferative vitreoretinopathy (PVR), posterior uveitis, RVO, retinal breaks, and retinal detachment. Vitreomacular traction (VMT) falls under the broader spectrum of vitreoretinal interface disorders, which also includes other conditions such as vitreomacular adhesion (VMA), ERM, full-thickness macular holes, lamellar holes, and pseudoholes [90]. VMA eyes were linked to a higher number of anti-VEGF treatments secondary to RVO [91]. Müller cells can generate stress fibers that provide adequate strength for retinal detachment in PDR [92]. In PDR, retinal detachment is connected to the emergence of two types of fibrotic tissue: fibrovascular proliferative tissue and avascular proliferative tissue, which may develop in PDR patients [92]. The presence of fibroblasts is essential in fibrosis in various retinal diseases. However, fibroblasts are absent in the CNS, and we believe that, in AMD, EMT may mediate the transformation of RPE cells into myofibroblasts that contribute to fibrosis. In

PDR, the role of fibroblasts is assumed by Müller cells, which play a critical role in PDR fibrosis along with astrocytes, microglia, and vascular cells [87]. Müller cells may further facilitate the progression of diabetic retinopathy. Another common factor is inflammation, which exacerbates fibrovascular scarring primarily through its role in angiogenesis. Angiogenic inflammatory cytokines such as interleukin-8 and tumor necrosis factor stimulate the proliferation of endothelial cells and promote angiogenesis, thus fostering diabetes-related retinal fibrosis [87]. CCN family member 2 (CCN2), also known as connective tissue growth factor (CTGF), is crucial in PDR pathogenesis, since the overexpression of the *CCN2* gene and the resulting increase in CCN2 levels in the vitreous have been reported [92]. CTGF promotes neovascularization and acts as a downstream mediator of TGFB2, a profibrotic cytokine, playing a significant role in fibrosis in AMD [93]. Additionally, CTGF has been linked to the pathological synthesis of periretinal fibrous tissue in the RPE of patients with AMD-related proliferative vitreoretinopathy [9]. Subretinal fibrosis in eyes with uveitis is a rare complication that typically occurs in the context of severe retinal detachment or chorioretinitis [94].

In summary, the fundamental mechanisms of subretinal fibrosis may be similar across various retinal diseases, but their underlying effects and factors can differ on the basis of the disease type. It seems that ETM or EndMT is a key mechanism in forming fibrotic scars, but it may also play a role in other conditions. TGFB2 could be a pivotal growth factor mediating fibrosis in all retinal diseases. Further studies are required to discern whether the fibrosis in neovascular AMD presents features distinct from those occurring in other retinal diseases, which could be targeted in antifibrotic therapy.

Autophagy in AMD

Cells remove damaged, dysfunctional, or unnecessary material, along with invaders and their remnants, through autophagy, which is a central molecular pathway essential for maintaining cellular and organismal homeostasis [95]. Autophagy can occur in two forms: degradative autophagy and secretory autophagy. In the former, the materials to be cleared are degraded within the cell and potentially recycled, while in the latter, they are expelled from the cell. Degradative autophagy is divided into three categories: macroautophagy (hereafter referred to as autophagy unless specified otherwise), microautophagy, and chaperone-mediated autophagy (CMA). Both autophagy and secretory autophagy begin with the formation of an isolation membrane (phagophore), which progressively surrounds the material to be degraded or removed until it is fully encapsulated, forming an autophagosome. This autophagosome then fuses with either the lysosome (in the case of degradative autophagy) or the plasma membrane (in secretory autophagy). The fusion of the autophagosome with the lysosome leads to the formation of an autolysosome (autophagolysosome), where degradation of the cargo occurs with the help of lysosomal enzymes. The products of this degradation may be recycled and utilized in cellular metabolism.

Microautophagy was the first autophagy pathway to be discovered, initially believed to be the sole constitutive autophagic pathway present in all cells [96]. In microautophagy,

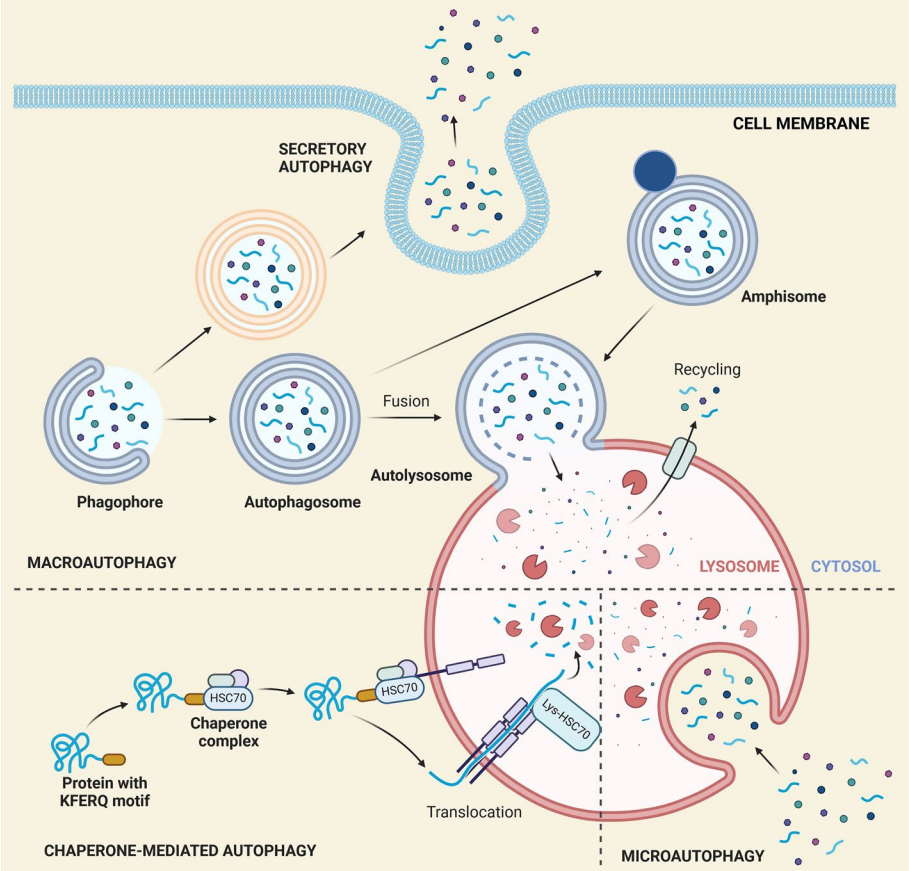


Fig. 5 Outline of degradative and secretory autophagy pathways. Both kinds of autophagy are initiated by the formation of the isolation membrane (phagophore), which grows to form an autophagosome to encapsulate the material to be degraded or secreted (cargo). Phagophore in secretory autophagy may be different from its degradative counterpart. In macroautophagy, the autophagosome fuses with the lysosome to form an autolysosome in which the cargo is degraded by lysosomal enzymes. A variant of macroautophagy may occur with the involvement of a late endosome (dark-blue circle) and the formation of an amphisome. In chaperone-mediated autophagy, the cargo carries a specific motif, exemplified here by a protein with the lysine–phenylalanine–glutamic acid–arginine–glutamine sequence recognized by a lysosomal membrane-bound protein with the involvement of a chaperone, exemplified here by heat shock cognate 71-kDa protein (HSC70). In microautophagy, the cargo is directly engulfed by a lysosome and degraded. In secretory autophagy, the autophagosome is different from its degradative counterpart and fuses with the plasma membrane to extrude the cargo. Created with BioRender.com

the cargo is nonselectively sequestered by direct engulfment in the deformations of the lysosomal membrane and is released into the lysosome for degradation (Fig. 5).

While macroautophagy and microautophagy nonspecifically degrade cellular waste, CMA selectively targets cytosolic soluble proteins for degradation. Selective recognition of cargo is mediated by cytosolic chaperones. Another essential difference between CMA and macro- and microautophagy is that substrates are not engulfed; instead, they are transported through the lysosomal membrane in a receptor-mediated manner [97] (Fig. 5). Currently, the heat shock cognate 71-kDa protein (HSC70) is the only chaperone demonstrated to be involved in CMA, but it collaborates with its co-chaperones, including HSC90 and HSC40 [97, 98]. CMA can be triggered by various factors, mainly

those that might induce conformational changes in the structure of cytosolic proteins [99].

Secretory autophagy may export proteins that lack a leader sequence, which is a 16–20-amino-acid segment at the N-terminus of certain eukaryotic proteins that determines their ultimate destination. Additionally, it may also play a role in extruding faulty cytoplasmic compounds, including mitochondria [100]. It may also take on the functions of impaired macroautophagy [101].

Autophagosome synthesis occurs through a group of four functional protein complexes, which includes the Unc-51-like autophagy activating kinase 1 (ULK1) complex, class III phosphatidylinositol 3-kinase (PI3KC3), two ubiquitin-like proteins, microtubule-associated protein 1 light chain 3 (LC3), autophagy-related protein 12 (ATG12), and the membrane cycling protein ATG9 [102]. The process begins with the nucleation of an isolation membrane (phagophore) and the activation of the ULK1 complex, which recruits ATG proteins. ULK1 then stimulates PI3KC3 by phosphorylating ATG14 and Beclin-1 (BCN1), thereby enhancing the membrane with phosphatidylinositol 3-phosphate (PI3P). ATG9 is translocated to the phagophore to supply lipids for its growth. ATG8 attaches to the phagophore through a reaction similar to E3 ubiquitin ligase, where the ATG12/ATG5/ATG16 complex conjugates ATG8 to phosphatidylethanolamine (PE) [103].

The fundamental machinery of degradative autophagy includes sequestosome 1 (SQSTM1/p62), optineurin (OPTN), ubiquilin 2, nibrin 1, WD repeat and FYVE domain containing 3, calcium-binding and coiled-coil domain 2, MTOR, and huntingtin [104]. The formation of autolysosomes through the fusion of autophagosomes with lysosomes is a hallmark of degradative autophagy and is mediated by soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins—a group that includes syntaxin 17, synaptosome-associated protein 29 (SNAP29), and vesicle-associated membrane proteins 7 and 8 [105]. That machinery targets its cargo through receptors/adaptors, which detect degradation signals on cargo proteins and bind to LC3 and γ-aminobutyric acid receptor-associated protein on the autophagosome [106].

Interleukins IL1B and IL18 were the first substrates identified for unconventional autophagy-based secretory pathways [107]. The secretory autophagy of IL1B begins with the formation of a complex involving the tripartite motif containing 16 (TRIM16) protein and its trafficking to an autophagy sequestration membrane [108], which is necessary for lipidation of LC3-I to LC3-II. Then, the IL1B–TRIM16 complex is associated with SEC22 homolog B vesicle trafficking protein (SEC22B), which possesses a domain crucial for protein transport to the plasma membrane and a SNARE motif essential for the fusion of the secretory autophagosome with the membrane. The R-SNARE, SEC22B, interacts with Qbc-SNAREs, SNAP23, and SNAP29 on the plasma membrane. STX3 and STX4 facilitate the formation of an SNARE complex on the plasma membrane that promotes the fusion of the secretory autophagosome with the plasma membrane and IL1B secretion.

The relationship between degradative and secretory autophagy remains unclear. Functionally, secretory autophagy may assume the role of its degradative counterpart when the fusion of the autophagosome with the lysosome is inhibited. Both types of autophagy may originate from common precursors. However, the secretory

autophagosome may differ significantly from the degradative autophagosome, as its primary function is trafficking and exporting extracellular cargo rather than degradation.

Impaired autophagy is a key factor in the pathogenesis of age-related macular degeneration (AMD), and its critical role in eye diseases is becoming increasingly evident [109]. Three recent reviews highlighted the significance of both degradative and secretory autophagy in the pathogenesis of AMD [25, 26, 110].

An increase in autophagosomes was observed in cultured human retinal pigment epithelium (RPE) from donors with age-related macular degeneration (AMD) compared with normal donors [111]. Furthermore, AMD RPE exhibited an accumulation of lipid droplets and glycogen granules, mitochondrial disintegration, increased susceptibility to oxidative stress, elevated levels of RONS under stress conditions, and diminished mitochondrial activity. Autophagy was impaired, and autophagic flux was reduced, as indicated by the lower ratio of LC3-II/LC3-I and the inability to downregulate SQSTM1 levels during starvation. Further investigations into impaired autophagic pathways revealed expanded and ring-shaped lysosomal associated membrane protein 1 (LAMP1)-positive organelles in AMD RPE, in contrast to the smaller discrete puncta noted in normal RPE. This research provided molecular insights into how impaired autophagy may contribute to AMD pathogenesis.

Dysfunctional mitochondria may generate increased levels of ROS, which are byproducts of normal mitochondrial function, as the complexes of the oxidative phosphorylation system (OXPHOS) produce ROS during their operation [112]. These ROS can damage biomolecules, including lipids, proteins, and nucleic acids, and autophagy may, at least in part, mitigate the effects of the harmful actions of these ROS. Therefore, autophagy may lessen the impacts of physical and chemical factors on the retina, acting protectively against the induction and progression of AMD. Studies have shown that autophagy activation induced by light pulses and phytochemicals counteracted oxidative stress in AMD [113]. These and other results led to the conclusion that autophagy, heterophagy, and mitophagy may provide protective effects against retinal damage and, therefore, prevent AMD [114]. On the other hand, impaired autophagy may contribute to AMD pathogenesis.

The promotion of autophagy and phagocytosis via the activation of the peroxisome proliferator-activated receptor alpha-transcription factor B/CD36 signaling pathway (PPARα-TFEB/CD36) has been shown to enhance RPE cell survival under oxidative stress in cell cultures and mouse AMD models [115]. We recently demonstrated that the AKT serine/threonine kinase 2/sirtuin 5/TFEB (AKT2/SIRT5/TFEB) pathway supports autophagy, enhances energy metabolism, and reduces the development of drusen, a key biomarker for the risk of developing neovascular AMD [116, 117].

Given the potential significance of autophagy in the pathogenesis of AMD, it is regarded as a therapeutic target for the condition (reviewed in Ref. [118]). Furthermore, in neovascular AMD, we can consider the dual effects of faricimab on VEGF and angiopoietin [119]. To support current intravitreal injections, we need additional targets that suppress active neovascular membranes. The regulation of autophagy signaling can potentially prevent fibrosis by enhancing energy metabolism. Despite active anti-VEGF treatment, some neovascular AMD patients develop

fibrotic scars and remain resistant to treatment, as shown in Fig. 2. Responses to anti-VEGF treatment may improve when active autophagy is maintained as a supportive activity [115, 116]. To prevent the progression of dry AMD, one might assume that it also stops the development of neovascular AMD. However, the recent effort to prevent the progression of geographic atrophy (GA), which targets the complement system, appears to be ineffective [4]. Autophagy and enhanced energy metabolism are considered important targets for additive therapy.

Selective autophagy refers to the lysosomal degradation of specific intracellular components that are sequestered into autophagosomes, late endosomes, or lysosomes. This process involves selective autophagy receptors, which interact with autophagy proteins through LC3-interacting region (LIR) motifs [120]. The deletion of the *ATG5* and *ATG7* genes was linked to an AMD-like phenotype in mice, characterized by RPE thickening, hyper- or hypotrophy, pigmentary abnormalities, and the buildup of oxidized proteins [121]. Oxidative stress is a consistently reported secondary risk factor for AMD and induces autophagy in RPE cells [122, 123]. AMD progression had a negative correlation with autophagic flux [123, 124].

Phagocytosis and recycling of the distal portions of photoreceptor outer segments are critical functions of RPE cells, as a single RPE cell contacts approximately 30 rods and cones [125]. These processes, known as heterophagy, necessitate an efficient lysosomal degradation pathway, which diminishes with age, contributing to AMD pathogenesis [126]. The autophagy receptor protein MTOR is a component of two complexes: mTORC1, which controls protein synthesis, cell growth, and proliferation, and mTORC2, which regulates the actin cytoskeleton and promotes cell survival and cycle progression. We showed that defects in the MTORC1 signaling pathway genes were linked to neovascular AMD [127].

Mitochondrial dysfunction plays a crucial role in AMD pathogenesis, and dysfunctional mitochondria are eliminated from cells through mitophagy, a specialized form of selective autophagy, to prevent the mitochondrial vicious cycle that leads to the overproduction of RONS [128]. Mitophagy involves numerous proteins, including PTEN induced kinase 1 (PINK1), Parkin RBR E3 ubiquitin protein ligase (PRKN), OPTN, autophagy and beclin 1 regulator 1, BCL2 interacting protein 3 (BNIP3), BCL2 interacting protein 3 like (BNIP3L/NIX), and FUN14 domain containing 1 [129, 130]. We observed an upregulation of PINK1 and PRKN, along with damaged mitochondria, in mice with double knockout of the nuclear factor erythroid 2-like 2 (NFE2L2) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A, PGC-1α) genes, resulting in an AMD-like pathological phenotype [131]. These results were confirmed in our later work, also demonstrating an upregulation of LC3B [132]. It was observed that impaired mitochondria were cleared independently of a conjugation system needed to conjugate ATG8 to phosphatidylethanolamine (PE) on autophagosome membranes, critical for macroautophagy [133]. Faulty mitochondria were expelled from cells through secretory autophagic pathways known as autophagic secretion of mitochondria (ASM). Since impaired mitochondria may directly contribute to the formation of lipofuscin and drusen, ASM could enhance mitochondrial quality control, which has declined in AMD [134].

In summary, there are many pathways through which autophagy may be involved in the pathogenesis of AMD. Despite consistent reports highlighting autophagy as an important factor in AMD pathogenesis, no ongoing clinical trials specifically target this process in AMD. Therefore, additional preclinical studies on the role of autophagy in AMD are necessary. However, autophagy is regarded as a "double-edged sword" in cellular homeostasis owing to its pro-life and pro-death activities [135]. Consequently, impaired autophagy might contribute to the pathogenesis of AMD, but the same concern may apply to overactive autophagy, which could degrade or extrude cellular components still necessary for homeostasis.

Impaired autophagy related to submacular fibrosis

Few experimental studies on the role of autophagy in retinal fibrosis could be found regarding AMD. Searching "autophagy fibrosis AMD" or "autophagy fibrosis age-related macular degeneration" in PubMed yields only one experimental paper demonstrating that the anti-fibrotic effects of 3-methyladenine (3-MA) in a mouse model of subretinal fibrosis were due to inhibition of the PI3K/AKT pathway instead of its PI3K-autophagy counterpart [136]. However, certain features of autophagy and the fibrotic process can be common across different organs, tissues, and cells. The transition of epithelial cells to a mesenchymal state may be a prerequisite for the initiation of fibrosis in many organs. Moreover, EMT stimulation may occur in these organs owing to shared factors, including growth factors.

As mentioned above, EMT is significant in fibrosis as it facilitates the transformation of RPE cells into myofibroblasts, which is crucial for fibrotic scar formation. Most studies examining the role of autophagy in EMT have focused on cancer, and this role is highly context dependent, meaning it varies on the basis of cancer type, stage of progression, type of cancer cells, and cellular environment. Although the formation of MNV and cancer progression necessitate angiogenesis, cancer cells exhibit many unique characteristics that cannot be directly associated with retinal cells. Therefore, findings from cancer research should not be directly applied to AMD. Moreover, despite substantial differences between advanced dry and neovascular AMD with systemic influence, they are both eye diseases.

Mouse embryonic fibroblasts (MEFs) from wild-type and transgenic animals serve as an established model for investigating autophagy in mesenchymal characteristic cells [137]. They can be combined with ARPE-19 cells to investigate EMT and its effects in the retina. ATG7 is an essential autophagy protein for autophagosome formation, the operation of the LC3 system, and ATG12 conjugation [138]. LC3-II was not detected in MEFs derived from ATG7 knockout mice, indicating that these cells cannot form autophagosomes and therefore are unable to perform autophagy [139]. The expression of SQSTM1 indicated a suppression of autophagic flux. The impairment of autophagy was linked to an increase in mesenchymal markers: N-cadherin, vimentin, and alpha smooth muscle actin, suggesting that autophagy deficiency could promote EMT in MEFs. Serum deprivation in WT MEFs, a condition that triggers autophagy, led to SQSTM1 degradation, LC3-II accumulation, and a reduction in N-cadherin. Therefore, autophagy inhibited the mesenchymal process. TGFB2 prompted the transdifferentiation of RPE cells into myofibroblasts, resulting in EMT. This process activated autophagy, and thus,

the lack of autophagy could facilitate EMT. Twist-related protein 1 (TWIST1) serves as the primary transcription factor in embryonic morphogenesis, activating mesenchymal markers and promoting EMT [140]. It was speculated that selective autophagy mediated by SQSTM1 might degrade TWIST1, opposing EMT. This speculation was confirmed by the observation of starvation-induced TWIST1 binding to SQSTM1, leading to the eventual degradation of TWIST1. However, TGFB2 stimulation did not induce TWIST1-SQSTM1 binding but rather blocked such binding when autophagy was active. Therefore, autophagy might prevent EMT induced by TGFB2. The production of stable ATG7 KD transfectants from RPE resulted in impaired autophagy, causing a loss of the epithelial phenotype and the acquisition of a mesenchymal phenotype by RPE cells. Consequently, functional autophagy was necessary to maintain the epithelial phenotype of RPE cells.

Treatment of ARPE-19 cells with TGFB2 and rapamycin, an autophagy activator, resulted in the inhibition of TGFB2-induced phosphorylation of MTOR, increased SQSTM1 degradation, and accumulation of LC3-II. Thus, rapamycin induced autophagy by blocking the phosphorylation of MTOR by TGFB2. Elevated autophagic activity protected RPE cells from EMT stress and inhibited their migration and contraction. Although this study was conducted in the context of proliferative vitreoretinopathy (PVR), a fibrous complication of intraocular surgery, the observed effects may lead to various outcomes, including retinal fibrosis in neovascular AMD [139].

Another study focusing on PVR mechanisms revealed the colocalization of keratin 8 (KRT8) with LC3B, an autophagy marker, in the subretinal and epiretinal membranes of patients with PVR [141]. Additionally, it was demonstrated that TGFB2 induced KRT8 phosphorylation and autophagy in ARPE-19 cells. Furthermore, these cells exhibited an upregulation of ETM and autophagy markers, indicating that TGFB2-induced ETM in RPE cells stimulated autophagy. As a result, blocking autophagy inhibited ETM in RPE cells, which was validated through both pharmacological and genetic studies. The varying conditions of both studies and the complexity of the autophagic process create challenges in directly comparing the outcomes of these two experiments.

Beta-crystallin A3 (CRBA1, βA3/A1-crystallin) plays an important role in lysosomal clearance and epithelial–mesenchymal transition (EMT) in retinal pigment epithelial (RPE) cells [142]. CRBA1 was upregulated in polarized RPE cells but not in undifferentiated cells. The loss of CRBA1 in murine and human RPE cells was linked to upregulation of snail family transcriptional repressor 1 and vimentin, downregulation of E-cadherin, and increased cell migration. Similar associations were observed in RPE cells isolated from samples of AMD patients compared with age-matched controls. The authors concluded that AMD might be initiated by defects in lysosomal clearance within the RPE and the subsequent epithelial–mesenchymal transition (EMT) of RPE cells to alleviate the stress associated with these clearance defects. Therefore, CRBA1 may be regarded as a target for AMD therapy to reverse EMT and prevent fibrosis.

Treatment of ARPE-19 cells with TGFB2 led to increased autophagic flux, as indicated by the expression of LC3-II and SQSTM1 [143]. Furthermore, autophagy activation enhanced the TGFB2-induced EMT, while autophagy inhibition led to EMT attenuation. Additionally, autophagy activation promoted the migration and invasion of RPE cells, whereas autophagy inhibition diminished these processes. The authors concluded that

autophagy might be a potential therapeutic target for reducing EMT in intraocular fibrotic disorders.

The extracellular matrix may play several roles in the pathogenesis of AMD [144]. RPE cells are situated between the neural retina and the choroid, and they are linked to the Bruch's membrane (BM)–ECM complex [145]. Damage to the RPE and choriocapillaris, along with inflammation in AMD, may result in the formation of an abnormal ECM [146]. Anomalous ECM results in altered RPE-choriocapillaris behavior, ultimately leading to atrophy of the retina, RPE, and choriocapillaris [147]. The imbalance between the production and removal of ECM components can result in the aggregation of ECM elements and their increased deposition, which may promote tissue fibrosis [148]. On the other hand, ECM components, including proteoglycans and active fragments, may either stimulate or inhibit autophagy [149–151]. Thus, the local environment of the retina, shaped by the composition of the BM–ECM complex, may affect the formation of retinal fibrotic scars not only by supplying collagen and other compounds essential to this process but also by modulating EMT mediated by autophagy.

The significant role of autophagy in regulating fibrotic processes has been demonstrated in various eye diseases. Indeed, 3-MA, an autophagy inhibitor, reduced the expression of fibrosis-related proteins fibronectin and collagen alpha-1(I) chain in the retinas of a mouse diabetic model [152]. However, treatment with chloroquine, another autophagy inhibitor, did not affect fibrosis or apoptosis-related proteins. Therefore, autophagy may regulate fibrosis in diabetic retinopathy, but further research is necessary to clarify the specifics of this regulation, particularly regarding the significance of autophagy phases (early versus late).

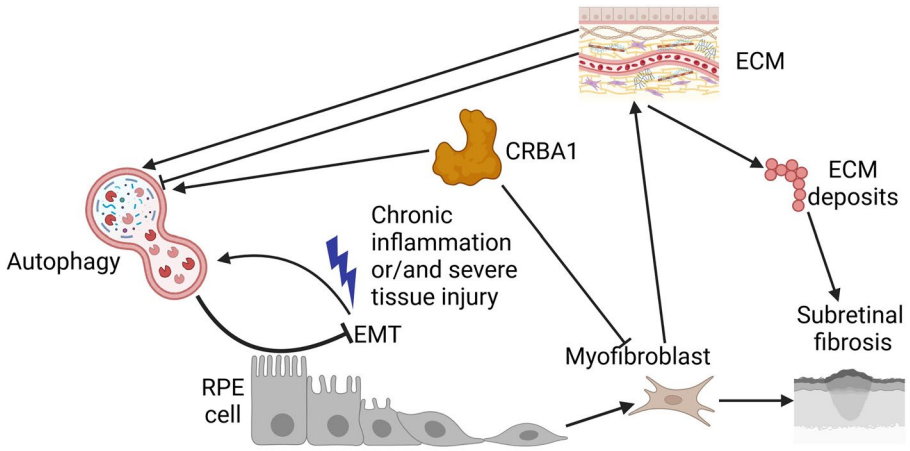


Fig. 6 Potential of autophagy in subretinal fibrosis in neovascular age-related macular degeneration (AMD). Retinal pigment epithelium (RPE) cells may be stimulated to undergo epithelial–mesenchymal transition (EMT) by low-level chronic inflammation or severe tissue injury, both typical for neovascular AMD, resulting in transdifferentiation to myofibroblasts, which support fibrosis. Extracellular matrix (ECM) may regulate autophagy, and dysfunctional ECM may deposit its proteins in a retinal scar. Myofibroblasts may change ECM composition and contribute to its dysfunctions. Beta-crystallin A3 (CRBA1) may positively regulate autophagy and support the epithelial phenotype of RPE cells, preventing myofibroblast transformation and resulting fibrosis. Signaling pathways regulating EMT are presented in Fig. 4. Subretinal fibrosis may also be promoted by the endothelial–mesenchymal transition of choroidal fibroblasts; retinal glial cells are not presented here for clarity. Created with BioRender.com

In summary, despite extensive literature on the role of autophagy in fibrosis related to cancer and pulmonary disorders, very few studies have explored the involvement of autophagy in fibrosis associated with AMD. Consequently, it is challenging to draw conclusions about the potential mechanisms behind this involvement. However, integrating experimental data for AMD with certain common aspects of autophagy and fibrosis allows us to infer some mechanistic features regarding the importance of autophagy in subretinal fibrosis (Fig. 6).

It can be assumed that the process occurring in the RPE, which ultimately results in subretinal fibrosis, begins with EMT, induced by various influences. While the growth factor TGFB2 is frequently used in experimental studies, many other factors may also trigger EMT in the RPE. Some of these factors could be associated with ongoing pathological processes in the eye, including AMD [153]. EMT may induce apoptosis if it has not already occurred, while autophagy inhibits EMT. When autophagy is impaired, the EMT of RPE cells may transform them into myofibroblasts, promoting fibrosis. Since ECM affects autophagy through various pathways, its abnormal functioning can lead to autophagy impairment. Furthermore, a dysfunctional ECM may deposit its components, such as collagens I and IV, fibronectin, and laminins at the site of fibrotic scar formation. In addition to RPE cells, choroidal fibroblasts and retinal glial cells may also transdifferentiate into myofibroblasts via EMT and EndMT, contributing to fibrosis. Regardless of their origin, myofibroblasts can synthesize and release ECM components, altering its content and functions, and thereby contributing to fibrotic scar formation [154]. This pathway involving autophagy in fibrosis needs empirical verification, as no experiments on the role of autophagy in the formation of subretinal fibrotic scars in neovascular AMD have been conducted so far.

As mentioned above, most studies on the autophagy–EMT relationship focus on cancer, and they conflict with findings in the retina. Unfortunately, we have not found any studies exploring this relationship in eye cancers, such as uveal/choroidal melanomas or retinoblastoma. Therefore, comparing studies on the autophagy–EMT relationship in cancer and noncancer tissues is challenging, even though both effects are underpinned by essentially the same mechanisms in cancerous and noncancerous cells. However, EMT in cancer cells is a marker of their neoplastic phenotype, as it facilitates invasion and metastasis, which are critical features of cancer transformation. Thus, the tendency to undergo EMT is inherent to cancer cells. In AMD, we cannot assert that EMT is an inevitable stage for every cell in the retina affected by AMD, as most cases do not develop a neovascular phenotype. The situation becomes more complex when autophagy is factored into these considerations. One immediate conclusion about autophagy is that it can act in a "pro-life" or "pro-death" manner, but these represent extreme conditions with numerous intermediate states [155]. Additionally, autophagy in cancer can have different effects under varying conditions. Cancer cells possess unique properties that regulate their proliferation through their own program, allowing them to proliferate despite natural anatomical and functional barriers. Even proliferative retinopathy, which is arguably the ocular disease most akin to cancer in nature, does not exhibit this type of program and is more similar to cancer angiogenesis than to the proliferation of cancer cells. Recently, a total of 5845 differentially expressed genes were identified between proliferative diabetic retinopathy patients and controls, including 24 EndMT-related marker genes and autophagy genes

[156]. It was also noted that anti-VEGFA treatment enhanced EndMT-related phenotypes. This work confirms the significance of autophagy and EndMT in retinal diseases but cannot be directly related to cancer research. Another disease extensively studied for EMT/EndMT–autophagy interactions is idiopathic pulmonary fibrosis [157]. Reports indicate that the inhibition of autophagy in alveolar epithelial cells promotes EMT, but it remains unknown whether autophagy is a "friend or foe" in IPF [157–159]. In summary, we assert that the inconsistencies in results regarding the role of autophagy in EMT in ocular and non-ocular tissues, particularly cancer, arise from the nature of the cells rather than from differences in research strategies and methodologies. Therefore, before understanding the nature of autophagy in specific cells and conditions, comparing results obtained in ocular and non-ocular tissues is a risky task.

Conclusions, outstanding questions, and perspectives

Retinal fibrosis may cause resistance to anti-VEGFA therapy and contribute to irreversible sight loss in patients with neovascular AMD. Therefore, it is particularly important to search for adjuvant therapies that can support anti-VEGFA treatment. A prerequisite is a better understanding of the molecular mechanisms underlying fibrosis in neovascular AMD. Epithelial–mesenchymal transition (EMT) of retinal pigment epithelium (RPE) cells and endothelial–mesenchymal transition (EndMT) of choroidal fibroblasts and retinal glial cells are crucial for the transdifferentiation of these cells into myofibroblasts, which are directly involved in forming fibrotic lesions. TGFB2 may be the master regulator of EMT/EndMT, but other proteins, including VEGFA, can also assume this role. Autophagy may be stimulated by EMT and subsequently inhibit it, though findings on the association between autophagy and EMT in the retina and other tissues are not all consistent. Therefore, future research should address the relationship between autophagy and EMT/EndMT.

In addition to EMT/EndMT, the abnormal functioning of the extracellular matrix (ECM) is essential for the formation of subretinal fibrotic scars, which contain several ECM components, including collagen and fibronectin. These ECM components also play a significant role in regulating autophagy, as several ECM-derived proteoglycans and proteins—including decorin, biglycan, endorepellin, endostatin, collagen VI, and plasminogen kringle 5—are known to induce autophagy [160]. However, some other ECM components, including laminin α2, perlecan, and lumican, suppress autophagy. The ECM can direct autophagy by interacting with numerous receptors and engaging with their co-receptors and adhesion molecules. Therefore, impaired ECM functions may lead to impaired autophagy.

Key events in the formation of subretinal scars include EMT/EndMT in RPE cells, choroidal fibroblasts, and retinal glial cells, along with abnormal ECM functioning that results in excessive deposits of its compounds in the outer retina. Autophagy in RPE cells may contribute to this process, as it may be stimulated by mesenchymal transitions and regulated by ECM components. However, it is not entirely clear how autophagy reacts to EMT/EndMT in the retina, as this may depend on the cellular context.

Low-level chronic inflammation and tissue injury may be primary factors initiating subretinal fibrosis. The former may serve as the source of growth factors and cytokines that stimulate EMT/EndMT (Fig. 4), and the latter may provide a basis for fibrotic scar

formation, as this process can be regarded as an aberrant form of wound healing. Both chronic inflammation and injury to the outer retina are characteristic of advanced neovascular AMD. Moreover, this disease is closely associated with dysfunctional ECM [161]. Therefore, events occurring in neovascular AMD may initiate fibrotic scar formation. At this point, two key questions arise. First, why do only some patients with neovascular AMD develop fibrosis, despite this advanced form of the disease being associated with extensive MNV? Second, while chronic inflammation and tissue injury are present in other retinal diseases and disorders of various eye structures, does neovascular AMD have distinct features compared with other eye diseases that promote fibrosis [162, 163]?

Autophagy plays a significant role in the pathogenesis of AMD and is reported to be altered in fibrosis across many organs [26, 164]. However, changes in autophagy cannot be definitively categorized as harmful or beneficial. Impaired autophagy may contribute to the pathogenesis of neovascular AMD and has been reported to have a protective effect against EMT in RPE cells. Consequently, neovascular AMD creates a pathway for EMT activation in RPE and fibrosis. Additionally, many aspects of autophagy remain without a molecular explanation, such as the interplay, if any, between degradative and secretory autophagy, which may be crucial for determining autophagy's role in the pathogenesis of AMD [25]. In the same context of removing components of the scar, microautophagy requires further investigation. Generally, many important questions regarding the role of autophagy in RPE cells and AMD need to be answered before accurately determining its potential in fibrotic scar formation in neovascular AMD.

The key role of TGFB2 in the formation of fibrotic lesions illustrates the significance of growth factors in this process (Fig. 4) and highlights their therapeutic potential in neovascular AMD. Research has shown that RBM-007, an aptamer targeting FGF2, inhibited FGF2-induced angiogenesis, laser-induced MNV, and MNV with fibrosis in animal models of neovascular AMD [72]. Therefore, compounds that exhibit anti-angiogenic and anti-fibrotic effects should be further explored as therapies, either independently or as adjuncts to anti-VEGFA treatment.

The resistance of neovascular AMD with fibrotic scar to anti-VEGFA therapy raises another question—can the process of intraocular injection of VEGFA antibodies contribute to the formation or progression of fibrotic scars? Studies conducted thus far do not unambiguously answer this question.

As fibrosis is explored as a potential therapeutic target in several diseases and causes some of the most serious consequences of neovascular AMD, it could be considered, alongside autophagy, in AMD therapy. However, preclinical studies and clinical trials are necessary to support this thesis.

Abbreviations

AKT	AKT serine/threonine kinase
AMD	Age-related macular degeneration
APOE	Apolipoprotein
ARMS2	Age-related maculopathy susceptibility 2
ASM	Autophagic secretion of mitochondria
ATG	Autophagy related
BM	Bruch's membrane
BNIP	BCL2 interacting protein 3
BNIP3L/NIX	BCL2 interacting protein 3 like
BRB	Blood–retina barrier

CRBA1	Beta-crystallin A3
CC	Choriocapillaris
CHF	Complement factor H
CMA	Chaperone-mediated autophagy
CNV	Choroidal neovascularization
CTGF	Connective tissue growth factor
ECM	Extracellular matrix
EMT	Epithelial–mesenchymal transition
EndT	Endothelial–mesenchymal transition
FGA	Fluorescein angiography
FGF2	Fibroblast growth factor 2
GA	Geographic atrophy
HSC70	Heat shock cognate 71-kDa protein
HTRA1	HtrA serine peptidase 1
KRT8	Keratin 8
IL1B	Interleukin-1 beta
IL8	Interleukin 8
IPF	Idiopathic pulmonary fibrosis
JAGGED/NOTCH	Protein Jagged1/2/neurogenic locus notch homolog protein 1
LC3	Microtubule-associated protein 1 light chain 3
MAPK	Mitogen-activated protein kinase
MNV	Macular neovascularization
MTOR	Mechanistic/mammalian target of rapamycin
NFE2L2	Nuclear factor, erythroid 2 like 2
OCT	Optical coherent tomography
OPTN	Optineurin
OVOL1/2	Putative transcription factor Ovo-like 1
PFGF, PFGFRB	Platelet-derived growth factor, PDGF receptor beta
PI3K	Phosphatidylinositol 3-kinase
PI3P	Phosphatidylinositol 3-phosphate
PINK1	PTEN induced kinase 1
PPARGC1A, PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-α
PRKN	Parkin RBR E3 ubiquitin protein ligase
PRRX1	Paired mesoderm homeobox protein 1
PVR	Proliferative vitreoretinopathy
RONS	Reactive oxygen and nitrogen species
RPE	Retinal pigment epithelium
RTK	Receptor tyrosine kinase
SEC22B	SEC22 homolog B vesicle trafficking protein
SHRM	Subretinal hyperreflective material
SMAD3	Mothers against decapentaplegic homolog 3
SNAI1/2	Zinc finger protein SNAI1/2
SNAP29	Synaptosome associated protein 29
SNARE	N-ethylmaleimide-sensitive factor attachment protein receptor
SQSTM1/p62	Sequestosome 1
TGFB2	Transforming growth factor beta-2 proprotein
TNF	Tumor necrosis factor
TRIM16	Tripartite motif containing 16
TWIST1	Twist-related protein 1
ULK1	Unc-51 like autophagy activating kinase 1
VEGFA	Vascular endothelial growth factor A
YAP	Transcription activator yes 1

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Author contributions
J.B. and K.K. conceived the review. J.B., E.P., H.H., M.D., M.I., and K.K. wrote the manuscript draft. J.B. and K.K. reviewed the manuscript. All authors read and approved the final version of the manuscript.

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