Myofibroblasts in macular fibrosis secondary to neovascular age-related macular degeneration - the potential sources and molecular cues for their recruitment and activation


Published in:
EBioMedicine

Document Version:
Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2018 the authors. This is an open access article published under a Creative Commons Attribution-NonCommercial-NoDerivs License (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits distribution and reproduction for non-commercial purposes, provided the author and source are cited.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.
Review

Myofibroblasts in macular fibrosis secondary to neovascular age-related macular degeneration - the potential sources and molecular cues for their recruitment and activation

Karis Little¹,a, Jacey H. Ma²,b,c, Nan Yang³, Mei Chen³, Heping Xu³,a,b,*

¹ The Wellcome-Wolfson Institute for Experimental Medicine, School of Medicine, Dentistry & Biomedical Sciences, Queen’s University Belfast, UK
² Aier Eye Institute, Aier School of Ophthalmology, Central South University, Changsha, Hunan, China
³ Guangzhou Aier Eye Hospital, Guangzhou, Guangdong, China

Abstract

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly in developed countries. Neovascular AMD (nAMD) accounts for 90% of AMD-related vision loss. Although intravitreal injection of VEGF inhibitors can improve vision in nAMD, approximately 1/3 of patients do not benefit from the therapy due to macular fibrosis. The molecular mechanism underlying the transition of the neovascular lesion to a fibrovascular phenotype remains unknown. Here we discussed the clinical features and risk factors of macular fibrosis secondary to nAMD. Myofibroblasts are key cells in fibrosis development. However, fibroblasts do not exist in the macula. Potential sources of myofibroblast precursors, the molecular cues in the macular microenvironment that recruit them and the pathways that control their differentiation and activation in macular fibrosis were also discussed. Furthermore, we highlighted the challenges in macular fibrosis research and the urgent need for better animal models for mechanistic and therapeutic studies.

Keywords:
- Age-related macular degeneration
- Macular fibrosis
- Myofibroblast
- Risk factors
- Inflammation

1 Introduction .............................................................. 284
2 Clinical features of macular fibrosis in nAMD .............................................................. 284
  2.1. Neovascular AMD .............................................................. 284
  2.2. Macular fibrosis in nAMD ..................................................... 285
  2.3. Risk factors of retinal fibrosis in nAMD ..................................................... 286
3 Pathophysiology of macular fibrosis .............................................................. 287
4 Molecular mechanism of macular fibrosis in nAMD .............................................................. 287
  4.1. The potential sources of myofibroblast precursors in macular fibrosis .............................................................. 287
    4.1.1. Glial cells .............................................................. 287
    4.1.2. RPE cells .............................................................. 287
    4.1.3. Endothelial cells ..................................................... 287
    4.1.4. Macrophages .............................................................. 287
    4.1.5. Circulating fibrocytes ..................................................... 287
    4.1.6. Choroidal stromal cells ..................................................... 287
  4.2. Molecular pathways involved in the recruitment of myofibroblast precursors .............................................................. 288
  4.3. Molecular pathways involved in the differentiation and activation of myofibroblasts .............................................................. 288
    4.3.1. Transforming growth factor-β (TGFβ) .............................................................. 288
    4.3.2. Platelet-derived growth factor (PDGF) .............................................................. 288
    4.3.3. Connective tissue growth factor (CTGF) .............................................................. 288
    4.3.4. Epidermal growth factor (EGF) .............................................................. 288
    4.3.5. Fibroblast growth factor (FGF) .............................................................. 288
    4.3.6. Sphingosine 1-phosphate (SIP) .............................................................. 289

* Corresponding author at: The Wellcome-Wolfson Institute for Experimental Medicine, Queen’s University Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK.
E-mail address: heping.xu@qub.ac.uk (H. Xu).

The authors contributed equally.
1. Introduction

Age related macular degeneration (AMD) is the progressive degeneration of the central part of the neuronal retina, the macula, in people older than 55 years of age, and is the leading cause of blindness in the elderly in industrialized countries [1]. Ageing, urbanization, obesity and cigarette smoke are risk factors for AMD [1]. Due to the growing ageing population and increased life expectancy, it has been projected that 288 million people will be affected by AMD by 2040 [2,3]. The early stage of AMD is characterized by large drusen and disturbance of retinal pigment epithelial (RPE) cells. The disease can advance into two late stages, “dry” and “wet”. Dry AMD refers to the geographic atrophy of the macula (Fig. 1A, B); whereas wet AMD is the growth of abnormal blood vessels into the macula (Fig. 1C, D), also known as neovascular AMD (nAMD). nAMD is treated by intravitreal injection of vascular endothelial growth factor inhibitors (e.g., anti-VEGF). Although the treatment can prevent the decrease or even improve vision acuity, it is burdensome, costly and invasive. Importantly, approximately one-third of patients do not benefit from the therapy due to the development of macular fibrosis or atrophy. The pathogenesis of macular fibrosis in nAMD is poorly defined, and currently there is no therapy for it. This review discusses the clinical manifestations and the underlying mechanisms of macular fibrosis secondary to nAMD with a strong focus on the potential source of myofibroblast precursors and the pathways involved in their recruitment and activation.

2. Clinical features of macular fibrosis in nAMD

2.1. Neovascular AMD

The growth of new blood vessels into the macula is the hallmark of nAMD. The new vessels cause leakage and hemorrhage beneath the RPE or photoreceptors resulting in severe visual loss. nAMD is classified into three subtypes according to the origin of diseased vessels, choroidal neovascularization (CNV) (Fig. 2A–D), retinal angiomatous proliferation (RAP) (Fig. 2E, F), and polypoidal choroidal vasculopathy (PCV) (Fig. 2G, H). CNV originates from choroidal blood vessels, which can be further classified into occult (CNV spreads beneath RPE cells, Fig. 2A, B) and classic (CNV breaks through RPE layer and grows in subretinal space, Fig. 2C, D) subtypes. RAP is the proliferation of retinal blood vessels that progress posteriorly into the subretinal space causing retinal-choroidal anastomosis (Fig. 2, E, F) [4]. RAP accounts for 15% to 20% of all nAMD cases in Caucasian population [4]. PCV is the polypoidal dilatations of an abnormal branching choroidal vascular network (BVN) (Fig. 2G, H), which can penetrate Bruch’s membrane and spread into subretinal space of the macula [5]. PCV is more prevalent than RAP in Asian populations [5].

![Fig. 1. Clinical features of late stages of AMD. (A, B) “dry” AMD (Geography atrophy), sharply circumscribed areas of RPE atrophy (black asterisks) associated scattered pre-existing drusen were seen in color photography (A). Loss of RPE (red arrows) and morphologic alteration of the outer retina (yellow asterisks) on SD-OCT B-scan (B). (C, D) “wet” AMD (nAMD). (C) Subretinal exudation and hemorrhage (black asterisk) were shown on the fundus photograph. (D) Fibrovascular lesion penetrates the RPE on SD-OCT (yellow asterisk). NL: Nerve fiber layer; GL: ganglion layer; ONL: outer nuclear layer; OLM: outer limiting membrane; EZ: ellipsoid zone; RPE: retinal pigment epithelia; Ch: Choroid.](image-url)
2.2. Macular fibrosis in nAMD

Macular fibrosis is the end stage of the natural history of nAMD [6,7]. It was originally described as disciform lesions by Pagenstecher in 1875, and was later found to be fibrotic scarring in histopathological examinations [8]. Macular fibrosis damages the RPE and photoreceptors resulting in irreversible visual impairment even with anti-VEGF agent treatment [9,10]. The incidence of macular fibrosis in nAMD was reported to range from 39% [11] to 100% [12] 10–15 years ago; and was decreased to 20·37–61·4% after the introduction of anti-VEGF therapy [9,13,14].

Clinically, macular fibrosis is defined as a well-dемarcated, elevated mound of whitish or yellowish material within or under the retina that

---

**Fig. 2.** Clinical features of different types of nAMD. (A, B) Occult CNV (type 1 CNV). Whitish hard exudation scattered around the swollen foveola (A). SD-OCT showed dome-shape elevation of intact RPE (B, red arrow) with intermediate hype-reflective mass (yellow asterisk). Bruch’s membrane was intact (white arrow). QLM: outer limiting membrane; EZ: ellipsoid zone; RPE: retinal pigment epithelia. (C, D) Classic CNV (type 2 CNV). Subretinal exudation (C, black asterisk) with fibrovascular lesion (D, yellow asterisk) penetrates the RPE (D, red arrow). The adjacent RPE contain subretinal hyper-reflective materials (SHRM, arrowheads). (E, F) Retinal angiomatosus proliferation (RAP), proliferating vessels within and below the retina. Retinal vessels (E, black arrows) connecting to the disciform scar. SD-OCT shows retinal vessel (F, blue arrow) insets into the sub-retinal hyper-reflective lesion (F, white arrows). (G, H) Polypoidal choroidal vasculopathy (PCV). Orange lesion (G, black asterisk) and subretinal hemorrhage were seen on color photograph. SD-OCT shows the thumb-like RPE detachment (H, green arrow) well correspond to polypoidal lesions, and the double layer sign (H, intermediate hype-reflective membrane located between RPE and Bruch’s membrane (white arrow)), which corresponds to the branched vascular network.
is not dehomoglobinized blood or hard exudation on fundus examination [13–15] (Fig. 3A). Fluorescein angiography (FA) usually demonstrates early hypofluorescent plaque masking the underneath (Fig. 3C) followed by non-leakage hyperfluorescein in the late stage (Fig. 3D). On spectral-domain optical coherence tomography (SD-OCT), fibrosis appears as a compact, subretinal hyper-reflective lesion, with aberrant RPE and ellipsoid zone (Fig. 3B, E) [15]. Subretinal fibrosis was classified into three stages using OCT and color fundus tomography [15]. Stage I: Minimal subretinal fibrosis – with a distinct pale patch lesion in fundus photograph and a thin continuous, highly reflective band on OCT between a grossly intact outer neurosensory retina and Bruch’s membrane, with or without subretinal fluid. Stage II: Prominent subretinal fibrosis – with a thick, continuous, highly reflective subretinal mass and diffuse thickening of the neurosensory fovea. Stage III: Hyper-reflective subretinal fibrosis – with atrophy of the underlying neurosensory retina [15]. On optical coherence tomography angiography (OCTA), abnormal vascular network and collateral architectural changes in the outer retina and the choriocapillaris layer are often evident, suggesting that the lesions are vascularized fibrotic membranes (Fig. 3E, F) [14].

2.3. Risk factors of retinal fibrosis in nAMD

AMD is related to several environmental and genetic risk factors. Age, smoking and diet are most consistently associated environmental risk factors of AMD [16]. Genetic studies have shown that the complement factor H (CFH) on chromosome 1 and the age-related maculopathy susceptibility 2/HtrA serine peptidase (ARMS2/HTRA1) genes on chromosome 10 have AMD susceptibility loci [17,18]. However, the environmental and genetic factors related to macular fibrosis in AMD have not been identified. Singh et al. reported that lower plasma levels of 25-hydroxyvitamin D is associated with subretinal fibrosis in nAMD [19]. The CFH Y402H CC genotype in nAMD patients is known to be related to poor response to anti-VEGF therapy, and patients with fibrosis are often poor responders [20]. This suggests that the CFH Y402H CC genotype may be a genetic risk factor.
for macular fibrosis, although direct evidence supporting the claim is lacking.

Among different types of nAMD, patients with RAP are more likely to develop macular fibrosis [21] than those with PCV [22]. Furthermore, classic CNV, poorer visual acuity at first presentation, a longer interval between diagnosis and treatment [13], refractory intraretinal cysts [23] and macular hemorrhage [24] are known risk factors of retinal fibrosis. SD-OCT investigations have shown that the presence of subretinal hyper-reflective material (SHRM, Fig. 2D) in RPE is related to macular fibrosis and poor vision prognosis [25]. In particular, the central location, a well-defined border and the thickness of SHRM can predict the likelihood of subretinal fibrosis [26]. The clinical observations suggest that damage to the neuronal retina and RPE put the macula at risk of developing fibrosis in nAMD.

3. Pathophisiology of macular fibrosis

Fibrosis is a pathogeological state of excess deposition of extracellular matrix (ECM) proteins in tissue repair. Wound healing occurs when injury to the tissue causes the recruitment and activation of inflammatory cells and fibroblasts. Activated fibroblasts (myofibroblasts) proliferate and deposit ECM proteins such as collagens, fibronectin, laminin and glycosaminoglycans. ECM spreads to cover and regenerate the damaged tissue. When repeated injuries occur or during chronic inflammation, this process becomes uncontrolled resulting in excessive fibrotic scarring. Macular fibrosis in nAMD has unique pathological features. First, it originates from a pre-existing neovascular membrane. Therefore, it is a conversion of the diseased vessels into fibrous tissues. Second, the fibrous tissue often has a vascular network (Fig. 3) i.e., a fibrovascular membrane [14].

The origin of myofibroblasts and the underlying mechanisms of ECM deposition in macular fibrosis remain poorly defined. It has been suggested that the macular scar may originate from retinal glial cells (e.g., Müller cells, microglia and astroglia) [27]. Histological studies in surgically removed CNV membranes (CNVMs) from nAMD patients have reported multiple cell types, including endothelial cells, RPE, macrophages and fibroblast-like cells [28]. Importantly, α-smooth muscle actin (α-SMA) positive cells, a characteristic marker of myofibroblasts have also been detected in CNVMs [29]. The results suggest that multiple cells may contribute to macular fibrosis.

4. Molecular mechanism of macular fibrosis in nAMD

The function of the myofibroblast is to synthesise and maintain ECM proteins. Since the retina and new blood vessels in nAMD do not contain fibroblasts, myofibroblasts in macular fibrosis must have been recruited or differentiated from other cells.

4.1. The potential sources of myofibroblast precursors in macular fibrosis

4.1.1. Glial cells

Müller glia and astrocytes provide framework support to retinal neurons and vasculatures. Under disease conditions, such as diabetic retinopathy and AMD, reactive Müller glia and astrocytes produce a variety of ECM and participate in retinal repair and remodeling [27]. Glial fibrillary acidic protein (GFAP) positive cells have been detected in an experimental model of subretinal fibrosis [30]. Under in vitro culture conditions, Müller cells lose the expression of glutamine synthetase and GFAP but gain the expression of αSMA suggesting that they have the potential to transdifferentiate into myofibroblasts [31]. Whether Müller cells can transdifferentiate into myofibroblasts in nAMD warrants further investigation.

4.1.2. RPE cells

RPE cells are known to contribute to retinal fibrosis, including macular fibrosis [32,33] through Epithelial-Mesenchymal Transition (EMT). The EMT is well-known as a contributing process in kidney, liver and lung fibrosis [34–36]. Clinical studies have observed higher incidence of fibrosis in classic CNV (compared with occult CNV) [13], in which the neovascular membrane penetrates the Bruch’s membrane-RPE complex into the subretinal space. Damaged RPE cells may become myofibroblasts through EMT and contribute to macular fibrosis. Various in vitro studies have shown that RPE cells can undergo EMT under experimental conditions [32,37]. Senescent cells can secrete a number of matrix metalloproteinases (MMPs) that are important for limiting fibrosis [38]. However, chronic presence of MMPs by senescent cells can damage tissue structure and function by inducing EMT [38]. Senescent RPE cells in nAMD may induce EMT by secreting MMPs.

4.1.3. Endothelial cells

Endothelial to mesenchymal transition (EndoMT) is known to contribute to organ fibrosis [39]. Endothelial cells of the neovascular membrane in nAMD are highly active, and they may transdifferentiate into myofibroblasts through EndoMT contributing to macular fibrosis. An EndoMT transcription factor, SNAI1 has been shown to critically contribute to the development of CNV [40]. The overall incidence of macular fibrosis has been reduced since the approval of intravitreal injection of VEGF inhibitors as a standard of care for nAMD. The results suggest that endothelial cells may contribute to macular fibrosis in nAMD.

4.1.4. Macrophages

Macrophages play an important role in tissue repair and wound healing [41]. A previous study has shown that 20% of the cells in experimental CNV were macrophages, and the majority (70%) of macrophages originated from the bone marrow [42]. During the early stages of inflammation, pro-inflammatory M1-macrophages produce cytokines to eliminate pathogens. After the inflammatory response subsides, M2-macrophages facilitate tissue repair by stimulating cellular proliferation and differentiation. Both M1 and M2 macrophages can participate in tissue fibrosis by secreting transforming growth factor – β (TGFβ) [43]. Later, macrophages may switch to a pro-resolving phenotype that suppresses the tissue-damaging inflammation and dissolves excessive ECM proteins [41]. When the pathogenic stimuli persist for a prolonged period, such as in AMD, the inflammatory and repair process may be dysregulated or maladapted leading to destructive fibrosis.

Macrophages can directly transdifferentiate to myofibroblasts, and this phenomenon is known to contribute to renal fibrosis [43]. Using lineage tracing studies, it was reported that the majority of macrophages undergoing MMT were CD206⁺ M2 macrophages [44]. Macrophages are capable of producing ECM proteins. Under in vitro culture conditions, TGFβ1 can induce macrophage expression of αSMA (Fig. 4), collagen 1 and fibronectin, suggesting that they can undergo Macrophage to Myofibroblast Transition (MMT).

4.1.5. Circulating fibrocytes

Circulating fibrocytes are a distinct population of blood-borne inactive mesenchymal cells that can enter tissue and become fibroblasts. They express the hematopoietic marker CD45, the progenitor marker CD34, and collagen. During tissue injury and inflammation, the levels of circulating fibrocytes are increased [45] and they may participate in wound healing and fibrotic tissue repair. Fibrocytes have been detected in CNVMs [28] and they may be an important source of myofibroblasts in macular fibrosis.

4.1.6. Choroidal stromal cells

Choroidal stroma contains melanocytes, macrophages, fibroblasts, lymphocytes and mast cells, as well as collagen and elastic fibres. Various choroidal stromal cells have been detected in the experimental model CNV and fibrosis [28]. A recent study in a mouse model of CNV/fibrosis has shown that choroidal pericytes can infiltrate the CNV and differentiate into collagen 1-expressing cells [46]. The choroidal stromal cells may be a potential source of myofibroblast precursors.
4.2. Molecular pathways involved in the recruitment of myofibroblast precursors

Clinical studies have shown nAMD eyes with persistent retinal and RPE disruption are more likely to develop fibrosis. This highlights the importance of retinal microenvironment in disease pathogenesis. A low-grade chronic inflammation is known to play an important role in AMD [47]. During inflammation, retinal cells (e.g., RPE and microglia) and infiltrating immune cells produce various cytokines and chemokines that may recruit myofibroblast precursors to the macula. For example, RPE, microglia and macrophages can all produce CCL2, a chemokine that plays an important role in the recruitment of CCR2+ macrophages [48] and fibrocytes [49]. CCR2+ macrophages critically contribute to the development of retinal neovascular membrane in laser-induced CNV [50] and chronic autoimmune uveoretinitis [51].

The cyclooxygenase-2 (COX-2) is also implicated in the recruitment of macrophages in the laser-induced CNV and selective inhibition of COX-2 suppresses CNV and subretinal fibrosis [52].

Fibrocytes express the chemokine receptors CCR2, CCR7 and CXCR4 that control their trafficking to sites of injury [53]. Different chemokine/chemokine receptor pathways are involved in fibrocyte trafficking under different pathological conditions. For example, in asthma, the CXCR4/CXCL12 axis critically controls fibrocyte infiltration during acute inflammation, whereas, the CCR7/CCL19 axis plays an important role in fibrocyte trafficking in chronic obstructive asthma [54]. In addition, the platelet-derived growth factor (PDGF) [55] and thymic stromal lymphopoietin [56] are also known to play a role in fibrocyte trafficking. The cells responsible for the recruitment of myofibroblast precursors in nAMD may include infiltrating macrophages, active endothelial cells and RPE. However, the underlying molecular pathways remain elusive.

4.3. Molecular pathways involved in the differentiation and activation of myofibroblasts

4.3.1. Transforming growth factor-β (TGF-β)

TGF-β is the ‘master regulator’ of fibrosis. The TGF-β family consists of around 30 members, and many of them signal via the SMAD signaling pathway. TGF-β has been implicated in the EMT [32,34] and MMT [44]. Previous studies have shown that the TGF-β-induced EMT in RPE cells involves the mitogen-activated protein kinases (ERK, p38 and JNK), SMAD2 and AKT pathways [57], which can be attenuated by activating the retinoic acid receptor-γ (RARγ) pathway [57]. EMT may also be induced by non-SMAD signaling pathways such as the wnt/β-catenin pathway [58]. Reactive Oxygen Species (ROS) is known to activate fibroblasts and increase collagen deposition in fibrosis through converting the latent form of TGF-β into its active form [59]. Oxidative stress is a major contributor of AMD, and ROS production by RPE or inflammatory cells may participate in macular fibrosis through activating TGF-β.

4.3.2. Platelet-derived growth factor (PDGF)

PDGF is a potent mitogen for mesenchymal cells such as myofibroblasts [60]. It also critically controls their migration and proliferation [60] and plays an important role in tissue repair, remodeling and organ fibrosis [60]. PDGF is critically involved in the pathogenesis of nAMD. A phase-2 clinical trial in nAMD using a dual antagonism of PDGF (Fovista) and VEGF (Ranibizumab) has observed significant benefits, including reduced macular fibrosis, compared with Ranibizumab monotherapy [61]. However, a follow-up phase-3 study (NCT01940900) did not confirm the improvement in visual acuity. Interestingly, a recent in vitro study has shown that a combined VEGF/PDGF inhibition induced a pro-fibrotic phenotype in human pericytes [62]. Further mechanistic studies may help to understand the role PDGF in retinal fibrosis.

4.3.3. Connective tissue growth factor (CTGF)

CTGF is an extracellular matrix-associated heparin–binding protein produced by many tissue cells including vascular endothelial cells [63], CTGF is critically involved in angiogenesis, tissue repair, and fibrosis through stimulating activation and proliferation of fibroblasts [64]. The role of CTGF in choroidal neovascularization [65] and retinal fibrosis [66] has been recognized for over a decade. A phase 1/2 trial with RXI-109, a self-delivering CTGF-RNAi for retinal fibrosis in nAMD has shown that the compound is well-tolerated when injected intravitreally (NCT02599064) although the therapeutic potential remains to be evaluated.

4.3.4. Epidermal growth factor (EGF)

EGF is a small polypeptide of 53 amino acid residues produced by many cells and is one of the best characterized epithelial cell mitogens [67]. EGF binds to its receptor EGFR resulting in cellular proliferation, differentiation, and survival [67]. Recent studies have shown that EGFR signaling is also critically involved in TGF-β-induced fibrotic gene expression [68]. Significantly higher levels of EGF have been detected in the aqueous humour of nAMD patients compared with cataract patients [69]. A recent study has shown that cigarette smoke-induced EMT in RPE cells is mediated through activation of the EGFR signaling pathway [70]. The role of EGF-EGFR signaling pathway in macular fibrosis warrants further investigation.

4.3.5. Fibroblast growth factor (FGF)

The FGFs are a family of cell signaling proteins consisting of at least 22 members and 4 receptors (FGFR1–4) [71]. The FGF-FGFR signaling responses are involved in diverse biological processes, including proliferation, migration, differentiation and apoptosis [71]. It has been reported that FGF2 is involved in the development of fibrosis in the bone marrow [72], lung [73], and retina [74]. Pharmacological inhibition...
of FGF2 in combination with VEGF inhibition is more effective in reduc-
ing CNV than inhibition of VEGF alone [74].

4.3.6. Sphingosine 1-phosphate (SIP)

SIP is implicated in the process of differentiation of fibroblasts to myofibroblasts and the EMT [75]. A previous study has shown that SIP is expressed at the lesion site in a mouse model of laser-induced CNV/subretinal fibrosis and intravitreal injection of anti-SIP monoclo-
nal antibodies reduces experimental CNV and retinal fibrosis [76]. SIP may play a role in macular fibrosis in nAMD.

4.3.7. Alpha B-crystallin

αB-crystallin is a major protein of the eye lens belonging to the small heat-shock protein family. αB-crystallin is also found in various non-
ocular tissues and acts as a molecular chaperone by preventing aggrega-
tion of proteins, inhibiting apoptosis and inflammation, and maintain-
ing cytoskeletal architecture [77]. αB-crystallin is reported to be in-
volved in tissue fibrosis including the retina [33]. αB-crystallin can in-
duce EMT and enhance TGβ3-induced EMT in RPE cells through the
SMAD4 pathway [33].

4.3.8. Galectin

Galectins are S-type soluble lectins that bind specifically to β-
galactoside sugars with intra- and extracellular functions. Galectins
are known to play critical roles in cell-cell adhesion, cell-matrix interac-
tions, macrophage activation, angiogenesis, metastasis, and apoptosis.
Previous studies have shown that galectin-3 is involved in TGβ3-
mediated myofibroblast activation [78] and increased plasma levels of
galectin-3 are associated with myocardial and vascular fibrosis [79]
and systemic sclerosis [80]. Although macular fibrosis in nAMD is a
vascularized fibrotic lesion, we did not detect any difference in the
plasma levels of galectin-3 between nAMD patients with and without
macular fibrosis and age-matched healthy controls (Yang et al., unpub-
lished observation). Galectin-1 was found to be upregulated in RPE in
the laser-CNV, and was reported to induce CNV-associated sub-retinal
fibrosis through EMT [81]. The role of galectins in macular fibrosis in
nAMD patients remains to be elucidated.

4.3.9. Decorin

Decorin is a proteoglycan that binds to collagen fibrils in the ECM. It
can interact with various growth factors and their receptors, includ-
ing TGFβ and participate in angiogenesis and fibrosis [82]. A recent
study has shown that increased decorin degradation is related to fibrotic
lung disorders [83]. Interestingly, we did not observe any difference in
the serum levels of decorin between nAMD patients and age-matched
controls. There was also no difference in serum levels of decorin be-
 tween patients with macular fibrosis and those without (Yang et al., un-
published observation). Whether the function of decorin is altered in
nAMD patients warrant further investigation.

4.3.10. Complement proteins

The complement system is an important arm of the innate immune
system, and dysregulated complement activation is known to be in-
volved in AMD [84,85]. Uncontrolled complement activation may con-
tribute to retinal pathology directly by damaging retinal cells using
the membrane attack complex (MAC, C5b-9) or indirectly by modulat-
ing inflammation with complement fragments e.g., C3a, C3b, and C5a
[84]. Previously, we reported that the plasma levels of C3a and C5a
were significantly increased in nAMD patients, particularly those with
macular fibrosis [86].

C5 polymorphisms have been reported in liver fibrosis [87]. Deletion
of C5 or blocking C5a receptor suppresses fibrosis in a mouse model
of late stage pancreatitis [88]. Furthermore, C3a can induce EMT in proxi-
mal tubular epithelial cells [89] and C3aR1 deletion inhibits interstitial
fibrosis in mice [89]. We have found that C3a can induce MMT (Little
et al. ARVO 2018 Abstract). C3a and C5a may participate in macular fi-
brosis in nAMD.

5. Therapeutic considerations

Macular fibrosis in nAMD develops from existing neovascular mem-
brane. If the neovascular membrane is eliminated at the very early stage
of nAMD, fibrosis is unlikely to occur. Unfortunately, the majority of
CNV lesions cannot be completely eliminated with existing anti-VEGF
therapy. Furthermore, ~30% of nAMD patients do not respond to anti-
VEGF therapy, and the reasons behind this remain unclear. Future stud-
ies may seek to understand this sub-set of patients, with aims to pro-
vide a preventative treatment strategy for those susceptible to macular
fibrosis.

When the neovascular lesion cannot be eliminated promptly, ap-
proaches should be taken to prevent fibrosis in conjunction with VEGF
inhibition. This may include inhibiting ECM production at the initia-
tion/progression stages and promoting ECM degradation and uptake
at late stages of fibrosis (e.g., the development of anti-fibrotic
macrophages).

Myofibroblasts are key for the development of macular fibrosis. Strate-
gies to prevent the recruitment of myofibroblast precursors or in-
hbit the activation or transdifferentiation of the precursors should ben-
efit nAMD patients. The challenge is to identify key molecules
responsible for the recruitment or the activation/transdifferentiation
of myofibroblast precursors. Among various growth factors discussed
here, only PDGF and CTGF have been tested in nAMD patients. Whilst
the therapeutic effect of CTGF inhibition remains to be evaluated, the in-
hibition of PDGF failed to show any additional benefit in patients with
VEGF inhibition in phase 3 trials. This highlights the complexity of the
disease mechanism and the need for further understanding of the path-
genesis of the disease.

6. Outstanding questions and challenges

Recent clinical studies have uncovered the morphological features
and the dynamic changes of the fibrotic lesion during different stages
of nAMD and following anti-VEGF therapy. However, the underlying
mechanism remains elusive. The pathogenesis of macular fibrosis in
nAMD is different from a normal wound-healing fibrotic scar as it arises
from a pre-existing neovascular membrane. The neovascular mem-
branes that originate from the retina (i.e., RAP) or invade the retina
from the chorioid (i.e., classic CNV) or those with refractory intraretinal
cysts are more likely to convert into fibrous lesions, highlighting the im-
portance of retinal microenvironment in disease process. Fibroblasts do
not exist in the macula, nor in the abnormal new blood vessels. Future
researches should aim to understand the sources of myofibroblast pre-
cursors, the molecular cues that attract them to macular neovascular
membranes and the pathways underlying their differentiation and
activation.

To address these important outstanding questions, we need to over-
come a number challenges. For example, laboratory examination of clin-
ically well-documented fibrotic samples will be extremely helpful to
understand the cellular and molecular base of clinical presentations
of macular fibrosis. However, as surgery is no longer a treatment option,
 Fibrotic macula can only be obtained through donations, and matching
membranes and the pathways underlying their differentiation and
activation.

Animal models are powerful tools to dissect disease pathways. Cur-
rently, rodents are most commonly used to study retinal fibrosis. As ro-
dents have no macula, the models cannot truthfully replicate the
mechanism of nAMD, fibrosis with macular fibrosis in patients. Neverthe-
less, they are valuable in dissecting specific pathways or testing drug candidates. For
example, the subretinal Matrigel implantation induced CNV and fibrosis
in rats has been used to test the therapeutic effect of VEGF traps [90].
The model may also be useful in testing anti-fibrosis drugs. Although
it can persist for 35 days [91], the subretinal fibrosis following the
laser-induced CNV, contains no blood vessels—a key characteristic of human macrovascular fibrosis in nAMD (Fig. 3) [29]. The model, therefore, has limited value in dissecting the molecular mechanism of macular fibrosis. The injection of peritoneal macrophages into the subretinal space immediately after the laser-induced CNV has resulted in a larger size of subretinal fibrosis [30]. The model has not been widely used due to the technical challenges (e.g., macrophages need to be injected precisely into the laser spot). Better animal models are urgently needed to understand the pathogenesis of macular fibrosis.

Acknowledgments

This work is supported by Fight for Sight (5057/5058), the Department for the Economy (DfE) of Northern Ireland, and National Natural Science Funds for Distinguished Young Scholar (NSFC 81500749).

Search strategy and selection criteria

Search strategy and selection criteria Data for this Review were identified by searches of MEDLINE, Current Contents, PubMed, and references from relevant articles using the search terms “neovascular AMD,” “macular fibrosis,” “optical coherence tomography,” “optical coherence tomography angiography,” and “myofibroblasts.” Abstracts and reports from meetings were included only when they related directly to previously published work. Articles published in English between 1875 and 2018 were included.

References

Samarakoon R, Dobberfuhl AD, Cooley C, Overstreet JM, Patel S, Goldschmeding R,
Chen M, Xu H, Parainflammation, chronic inflammation, and age-related macular
inflammatory macrophage subpopulation critical for angiogenesis in tissue repair.
Moore BB, Murray L, Das A, Wilke CA, Herrygers AB, Toews GB. The role of CCL12 in
the recruitment of fibrocytes and fibroblasts. Am J Respir Cell Mol Biol 2006;35(2):
175–81.
Sakurai E, Anand A, Ambati BK, Van Rooijen N, Ambati J. Macrophage depletion in
hibits experimental choroidal neovascularization. Invest Ophthalmol Vis Sci 2003;
44(8):3578–85.
Chen M, Copland DA, Zhao J, Liu J, Forrester JV, Dick AD, et al. Persistent inflamma-
tion subverts thrombospondin-1-induced regulation of retinal angiogenesis and is
Zhang R, Liu Z, Zhang H, Yin L. The COX-2-selective antagonist (NS-398) inhib-
e0146808.
Reikofka RA, Buckala R, Herzog EL. Fibrocytes: emerging effectors cell in chronic in-
trafficking in patients with chronic obstructive asthma and during an acute exacerbation.
derived growth factor/platelet-derived growth factor receptor axis in the traffick-
ing of circulating fibrocytes in pulmonary fibrosis. Am J Respir Cell Mol Biol 2014 Dec;
contribution to the recruitment of circulating fibrocytes to the lung in a mouse
RPE cells and subretinal fibrosis by a RAR–? agonist. J Mol Med 2015;93(7):
749–58.
Wei Z, Caty J, Whitson J, Zhang AD, Srinivasagan R, Kavanagh TJ, et al. Reduced glu-
ochon level promotes epithelial-mesenchymal transition in lens epithelial cells via
a Wnt/beta-catenin-mediated pathway: relevance for cataract therapy. Am J Pathol
2018;184(1):165–79.
Morry J, Ngamcherttrakul W, Yantasee W. Oxidative stress in cancer and
He S, Jin ML, Worpel V, Hinton DR. A role for connective tissue growth factor in the
Kuker EJ, de Smet MD, van Meurs JC, Tan HS, Tanck MWT, Oliver N, et al. Induction of
fibroblast growth factors by TGF-beta1 requires EGFR activation, p53 and reactive
Jonas JB, Jonas RA, Neumaier M, Findsenes P. Cytokine concentration in aqueous