# Chapter 19
# Perspectives in New Advances in Retinal Neovascularization Pathogenesis and Therapeutic Approaches

**Temitope Sasore and Jian-Xing Ma**

**Abstract** Ocular neovascularization (NV) is the primary cause of catastrophic loss of vision in vast majority of ocular diseases including age-related macular degeneration, proliferative diabetic retinopathy and retinopathy of prematurity. The development of abnormal blood vessels in these patients is driven by a complex signaling process involving pro-angiogenic mediators such as vascular endothelial growth factor (VEGF) and anti-angiogenic factors, such as pigment epithelium-derived factor. Current anti-VEGF drugs such as ranibizumab, aflibercept and "off-label" bevacizumab are effective in only 30–40% of patients and are typically associated with undesirable route of administration, increased risk of infection and high clinical costs. This therefore increases the urgency to discover and develop additional therapeutics that are safer and more efficacious. In the last few years, several studies have contributed to understanding the underlying pathogenesis of ocular NV and the roles of different signaling cascades. Thus, this article aims to review molecular mechanisms regulating ocular NV and emerging therapeutic strategies to treat this group of diseases.

**Keywords** Angiogenesis • Inflammation • Vascular endothelial growth factor (VEGF) • Retina • Choroid • Ocular neovascularization (NV) • Age-related macular degeneration (AMD) • Diabetic retinopathy (DR) • Choroidal neovascularization (CNV)

T. Sasore • J.-X. Ma (✉)
Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Harold Hamm Oklahoma Diabetes Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA
e-mail: jian-xing-ma@ouhsc.edu



J.L. Mehta et al. (eds.), *Biochemical Basis and Therapeutic Implications of Angiogenesis*, Advances in Biochemistry in Health and Disease 6,
DOI 10.1007/978-3-319-61115-0_19

## 1 Introduction

Ocular neovascularization (NV), the final common pathway seen in ocular disorders such as age-related macular degeneration (AMD), proliferative diabetic retinopathy (PDR), retinopathy of prematurity (ROP), occlusive retinal vasculopathies and other ocular inflammatory diseases is characterized by abnormal or excessive in-growth of blood vessels, a process known as angiogenesis. Together, these debilitating eye complications represent the leading causes of human blindness and remain a huge socioeconomic burden for health care systems and patients worldwide. For example, recent research by the National Eye Institute estimates that the number of expected cases of AMD will increase from ~2 million to over 5 million by the year 2050 (https://nei.nih.gov/eyedata/amd). The burden placed by vision loss, not only on the healthcare system, will continue to grow unless greater steps are taken to understand and treat eye conditions that cause vision impairment. The "common denominator" shared by the aforementioned diseases is the excessive growth of unwanted vessels either at early or later stages of life, which often lead to vision impairment and blindness. Over the years, researchers have successfully mimicked some of these ocular pathologies in animal models, for example the oxygen-induced retinopathy (OIR) model to study ROP, and the laser-induced choroidal neovascularization (CNV) to study AMD [1, 2]. The understanding of these neovascular-related complications led to the identification of the well-recognized anti-vascular endothelial growth factor (VEGF) molecules, ranibizumab and aflibercept as treatment strategies [3]. However, the limitations of these drugs include high costs, limited population of responders (over two-thirds of patients fail to respond), an invasive route of administration and adverse drug reactions certainly calls for identification of improved therapies [4]. Thus, this review will discuss the current and emerging therapies for the treatment of ocular NV.

## 2 Ocular Neovascularization and Related Eye Diseases

Under normal physiological milieu, angiogenesis is tightly regulated by a stringent balance between pro-angiogenic factors, such as VEGF and anti-angiogenic molecules such as pigment epithelium-derived factor (PEDF). In contrast, excessive blood vessel growth is usually preceded by an imbalance between both pro- and anti-angiogenic molecules [5], consequently giving rise to a wide range of vascular diseases such as AMD, PDR, RVO, and ROP. Together, these ocular complications make up the leading causes of irreversible blindness and visual impairment worldwide.

## 2.1 Age-Related Macular Degeneration

AMD, the most prominent form of vision loss affecting elderly individuals aged over 50, is an ocular condition caused by defective function of the retinal pigment epithelium (RPE) which in turn leads to the development of degenerative lesions in central region of the retina, known as the macula. Located in the macula, are specialized photoreceptors cells responsible for detailed and sharp focus. As such, the breakdown of these light-sensitive cells in patients with AMD results in a gradual and steady loss of central vision. Clinically, AMD can be diagnosed as either "dry" AMD or "wet" AMD. Dry AMD, also known as non-exudative or atrophic AMD, is the most common form and occurs in 80–90% of AMD patients [6]. This is usually characterized by the accumulation of ophthalmoscopically visible yellow deposits known as drusen between the RPE and the Bruch's membrane [7]. It is understood that the presence of localized drusen ("soft" or "hard") is the result of undigested material from dysfunctional phagocytic cells that increases with aging and accumulates in the RPE [8, 9]. Typically, dry AMD begins with its early stages featuring a few drusen deposits causing slight blurred vision [9]. However, this can then progress slowly to a more advanced dry AMD (without turning into the wet form) where drusen deposits grow in size. This classic feature of late stage dry AMD causes breakdown or damage of light-sensitive retinal cells (atrophy) and as a result leads to loss of central vision.

Dry AMD can sometimes progress to wet AMD, also known as exudative or neovascular AMD, which is more severe but less prominent as it occurs in 10–20% of all AMD patients [10, 11]. In wet AMD, there is an abnormal growth of blood vessels from the choroid layer through the Bruch's membrane and into the macula, a process known as choroidal neovascularization (CNV) [12]. As these vessels are fragile, they often leak blood content and fluid into the retina, therefore leading to damage of light-sensitive cells and scarring of the macula. These pathological features usually result in the classic hallmark; presence of blind spots and loss of central vision. Wet AMD is responsible for ~90% of severe visual loss in AMD [12]. Interestingly, it is possible to experience both forms of AMD at the same time, in one or both eyes. In addition, the onset and progression of either type does not follow any particular pattern.

## 2.2 Proliferative Diabetic Retinopathy and Diabetic Macula Edema

DR reflects disruptions of the retinal vasculature resulting from elevated blood glucose. In addition to chronic hyperglycemia, there is evidence suggesting hyperlipidemia and hypertension also contribute to the development of DR [13]. Characteristic pathologies of this disease include pericyte loss, basement membrane changes, microaneurysms (vessel wall swelling), capillary occlusion, vascular leakage or blood retinal barrier breakdown and retinal NV [14]. DR is commonly classified

into non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) [15]. The former, characterized by presence of hard exudates, vascular leakage and aneurysm, is subdivided into mild, moderate and severe stages. PDR on the other hand, is a more advanced stage of the disease and is characterized by the development of abnormal retinal microvessels (retinal NV).

These proliferative changes, in most cases, trigger the onset of macular edema (DME) whereby the fragile and thin vessels can leak fluid into the macula, causing it to swell [15]. DME can develop at any stages of DR but mostly occurs as the severity of DR increases. PDR and DME, the two sight-threatening features of diabetes, make up the most prevalent causes of blindness and visual impairment amongst working-age individuals (20–65) of most developed countries [16].

### *2.3 Retinopathy of Prematurity*

Retinopathy of Prematurity (ROP) is a common vasoproliferative disorder of the retina and a major cause of blindness in ~50,000 premature infants of developed countries [17]. In the normal human fetus, the development of retinal vasculature occurs in-utero and commences from approximately 16th week of gestation till the 40th week of gestation, where the eyes become fully vascularized. However, preterm delivery of infants results in incomplete vascularization of the retina and as a consequence predisposes the immature retina to debilitating complications. ROP can be classically segregated into two distinct phases, consisting of phase I whereby initial retinal vessel growth is ceased and secretion of pro-angiogenic factors, such as insulin-like growth factor-1 and VEGF, is down-regulated at the time of preterm birth [18]. As development continues, the avascular retina becomes increasingly hypoxic therefore triggering increased metabolic activity. In phase II of ROP, the hypoxic condition from the prior stage stimulates the secretion of pro-angiogenic factors and as such triggers retinal NV as in other proliferative retinopathies [19]. With increasing severity, this second stage progresses as an uncontrolled fibrovascular proliferation into the vitreous and ultimately leads to tractional retinal detachment and the associated blindness [20].

## 3 Angiogenesis

In the developing mammalian embryo, vascular development occurs via two distinct, yet interrelated processes termed, vasculogenesis and angiogenesis [21]. The former involves differentiation of mesodermal cells into hemangioblasts. The peripheral hemangioblasts then differentiate into endothelial precursor cells, angioblasts, leading to the formation of tube-like endothelial structures [22]. The latter on the other hand, is characterized by the subsequent sprouting and remodeling from pre-existing vessels into a mature vascular network [23]. Altogether, vascular

development is typically an essential requirement for biological processes such as wound healing, organ development and the female reproductive cycle.

Similar to other organs, development of the mammalian eye is influenced by the intense process of angiogenesis which is necessary for the oxygenation of ocular tissues [24, 25]. The molecular basis of angiogenesis is characterized by an orderly cascade of complex events regulated by angiogenic molecules and degrading enzymes [26]. The release of pro-angiogenic factors such as VEGF, fibroblast growth factor and angiopoietin 2 are largely in response to hypoxia or other endogenous stimuli. Upon their release, these proangiogenic factors bind to surface receptors of neighboring vessels, thereby promoting endothelial cell activation [27]. Subsequently, enzymes such as matrix metalloproteinases are secreted by activated endothelial cells. These extracellular proteases are responsible for the degradation of the basement membrane. Therefore, this allows the proliferation and migration of endothelial cells towards angiogenic stimuli such as VEGF. The proliferating cells connect with nearby endothelial cells, and specific adhesion molecules, such as intergrins (αvβ3, αvβ5) are released to accommodate cell migration and neovessel sprouting [28]. As the sprouts elongate, proliferating endothelial cells are reorganized to form tube-like structures with a central lumen. Each individual blood vessel tube buds with adjacent tubes, thereby producing a functional vascular network capable of circulating blood. Additionally, pericytes and smooth muscle cells are then recruited to stabilize the newly formed vessels [27].

## 4 Mammalian Ocular Angiogenesis and the Role of VEGF in Mammalian Retina

The mammalian retina receives its nutrition from two discrete circulations, the retinal and choroidal circulations [29, 30]. The choroidal and hyaloid vessel which conducts ~80% of retinal circulation nourishes the outer retina, to ensure the oxygenation of the retina during the initial development of the eye as the inner retinal vasculature is absent [29]. In contrast, the remaining ~20% circulation is carried by the central retinal artery emerging from the optic nerve head, to nourish the inner retinal layers during the late eye development [24]. During development, the mammalian ocular vascular network undergoes key physiological changes.

The matured retinal vasculature is made up of two laminar layers: the primary superficial layer and the deep vascular layer involved with the development of astrocytes and Müller cells, respectively. By virtue of its induction by hypoxia-inducible factor 1, a transcription factor which binds to the hypoxia responsive element in the promoter region of the VEGF gene, VEGF is the principal mediator needed for stimulating retinal vascular development [31]. Studies by Miller et al. and Alon et al. report a correlation between the spatial and temporal changes in VEGF mRNA levels in a rat model of retinal ischemia [32, 33]. Furthermore, Aiello et al. also assess the anti-angiogenic effect of VEGF-neutralizing proteins in a mouse OIR

model. Here, authors report the human Flt or murine Flk chimeric protein resulted in complete inhibition of retinal NV in treated mice [34]. Ozakia and colleagues demonstrated that PTK787, a VEGF inhibitor, blocked the phosphorylation of VEGF, completely inhibited retinal NV in a murine OIR model and partially inhibited retinal vascularization during development, therefore suggesting that VEGF plays a vital role in retina NV [35].

## 5 Current Therapies for Ocular Neovascularization

The importance of ocular NV is crucial to the pathology of the aforementioned ocular complications, with growth factors such as VEGF implicated in the disease process. As such, therapeutic targeting of VEGF in the posterior eye has been a central focus for the treatment of these diseases.

### *5.1 Vascular Endothelial Growth Factor Inhibitors (Anti-VEGFs)*

Pegaptanib (Macugen®; Eyetech, Palm Beach Gardens, FL) a ribonucleic acid aptamer directed against the VEGF165 isoform, was the first anti-angiogenic therapy approved for neovascular AMD in 2004 [36]. Bevacizumab (Avastin®; Genentech/Roche, San Francisco) is a full-length, humanized monoclonal antibody that binds to all VEGF-A isoforms. In 2004, Avastin was approved exclusively for the treatment of metastatic colon cancer and often used as off-label to treat ocular NV following its tolerability and efficacy evaluation [3]. In 2006, in an effort to improve retinal penetration and systemic half-life, Ranibizumab (Lucentis®, Genentech, San Francisco), a corresponding Fab fragment of full-length Bevacizumab was specifically designed and approved by FDA for treatment of CNV due to AMD [37]. It is a humanized, recombinant, monoclonal antibody Fab fragment which binds and neutralizes all identified VEGF-A isoforms. Aflibercept (Eylea® (VEGF Trap-Eye), Regeneron), which was approved by FDA in 2011 for treatment of exudative AMD, is a humanized, recombinant VEGF-receptor fusion protein that binds to all forms of VEGF-A, VEGF-B and the associated placental growth factor with high affinity, thereby preventing activation of cognate VEGF receptors [38].

Table 19.1 summarizes the features of current anti-VEGF drugs.

**Table 19.1** Molecular characterization of select FDA-approved anti-VEGF drugs

| Name | Molecular weight (KD) | Half-life (days) | Binding specificity | Fc fragment | Structure components |
|---|---|---|---|---|---|
| Pegaptanib | 50 | 10 | VEGF-164/165 | No | Pegylated oligonucleotide aptamer |
| Bevacizumab | 149 | 5.6 | VEGF | Humanized IgG | Full length humanized anti-VEGF monoclonal antibody |
| Ranibizumab | 48 | 3.2 | VEGF-A | No | Humanized monoclonal antibody with only Fab |
| Aflibercept | 115 | 4.8 | VEGF-A, VEGF-B, PIGF | Human IgG | Chimeric receptor comprised of the second Ig domain of VEGFR-1, the third Ig of domain VEGFR-2 in the Fab, and a human IgG fc |

## *5.2 Photodynamic Therapy (PDT) and Laser Photocoagulation*

Besides the broadly used anti-VEGF's, visudyne photodynamic therapy (PDT) and laser photocoagulation represent other therapeutic strategies for the clinical management of ocular NV [39]. PDT is a two-step procedure whereby a pharmacological photosensitizer (e.g. verteporfin (Visudyne®)) is first administered intravenously, followed by its subsequent activation using a laser light. This visible light induces a photo-oxidative damage of vascular endothelium, thereby selectively destroying unwanted retinal vessels. However, PDT appears to stimulate the release of VEGF and other inflammatory mediators [40], an initial problem in ocular NV. Thus, combination of PDT therapy with an intravitreal steroid (e.g. triamcinolone acetonide) or anti-VEGF (e.g. ranibizumab) adjunct is increasingly being studied and applied to inhibit the expression of VEGF and other inflammatory mediators [41]. Laser photocoagulation, on the other hand, uses laser burns to directly reduce retina vessel leakage or, in some case, destroys tissue in the peripheral retina, therefore reducing oxygen demand and alleviating ischemia in central retina. These procedures are often used to halt disease progressing to a more serious condition such as PDR and DME.

## *5.3 Limitations*

Typically, these anti-VEGF biologicals are administered through intravitreal injections into the vitreous of the patients' eye. Despite their therapeutic benefits in some AMD patients, long-term visual improvements of anti-VEGF therapies are impeded in 60–70% of patients due to sub-optimal dosing, genetic variations, rapid drug

clearance, tachyphylaxis and poor access to clinics [4, 38, 42]. As such, patients usually require monthly in-clinic injections in order to obtain significant therapeutic efficacy. Owing to their invasive route of administration, this classic treatment modality is associated with potentially severe complications including increased risk of infectious endophthalmitis (up to 1.6% occurrence following intravitreal anti-VEGF injection) [43], ocular hemorrhage (up to 10% occurrence) [44]; intraocular inflammation (1.4–2.9% occurrence) [45], retinal detachments (less than 1%) [46] and not to exclude the enormous yearly cost of ranibizumab and aflibercept.

Although the application of verteporfin PDT and laser photocoagulation are less common, the major drawback of these treatment strategies is that they can entail small retinal scars which can cause blind spots in patients' field of view and may induce vision loss [41]. A complementary approach to circumvent these drawbacks is to engineer the development of VEGF-independent molecules that are more efficacious and could be delivered topically or as sustained release implants, therefore reduce the frequency of intravitreal injections.

## 6 Emerging Therapies to Treat Ocular Neovascularization

Aside from the anti-VEGF drugs, there are several potential treatment strategies emerging through the pipeline and hold promise for improving treatment of ocular NV. These include endostatin, PDGF inhibitors, PEDF, integrin receptor blocker, complement cascade inhibitors, gene therapies and anti-immune/inflammatory molecules.

### *6.1 Endostatin*

Endostatin, another endogenous inhibitor of angiogenesis, has been demonstrated to have significant inhibitory effect on retinal NV [47]. Here, authors showed that endostatin prevented endothelial cell migratory and tubular network formation processes, as well as the secretion of VEGF in endothelial cells. Moreover, intraocular injection of endostatin convincingly reduced neovascular areas in mouse OIR model. However, as endostatin is unstable in properties and is unable to penetrate through the BRB, efforts are being made to improve the permeability of endostatin. Recently, Li et al. used a genetic engineering method to fuse Tat PTD, a protein transduction domain of the Tat protein of HIV-1, with endostatin. The successful generation of Tat PTD-endostatin (Tat PTD-Es) not only resulted in increased ocular barrier penetrance following topical administration, but also maintained inhibitory effects on CNV [48]. Tat PTD-Es has been modified by the introduction of a tripeptide of arginine-glycine-aspartic (RGD) to its structure, which improves its binding specificity to $\alpha_v\beta_3$ integrin that is highly expressed on endothelial cells in

pathologic conditions [49]. Tat PTD-Es-RGD similarly demonstrates high BRB permeability and inhibits abnormal retinal angiogenesis and therefore could offer an innovative therapeutic option for the prevention of retinal NV through eye drop formulations.

## 6.2 Pigment Epithelium-Derived Factor

Pigment epithelium-derived factor (PEDF), a glycoprotein secreted by most cells, is well understood to have neurotropic and anti-angiogenic activity in mammalian retina [50]. As a potent endogenous inhibitor of ocular angiogenesis, PEDF halts the development of neo-vessels by inducing apoptosis of endothelial cells activated for new vessel formation [51]. Studies reporting decreased PEDF levels in the vitreous, aqueous humors and retinas of PDR-affected eyes highlight the importance of PEDF to human blindness [52, 53]. As several ocular NV pathologies are characterized by neuronal loss, PEDF presents as an attractive therapeutic protein as a result of its multifunctional activity.

Emerging as a therapeutic strategy, Mori et al. demonstrated that adenovirus-mediated gene transfer of human PEDF by intraocular injection destabilized CNV in mouse eyes [54]. Amaral and Bacerra also reported that PEDF34-mer (Asp(44)-Asn(77)), a functional PEDF N-terminal peptide, exerted significant PEDF-like anti-angiogenic effect in a rat model of laser-induced CNV [55]. According to the study, subconjunctival administration of 0.1 and 1 pmol/d of the synthetic peptide dose-dependently reduced CNV lesion volumes compared to vehicle [55]. Furthermore, supporting evidence has also shown that PEDF over-expression delays photoreceptor and neural retinal cell death – a contributing factor in retinal diseases [56]. Conversely, it has been postulated that increased circulatory PEDF in Type 1 and Type 2 diabetes patients may exacerbate systemic symptoms of diabetes such as impaired wound healing due to impaired peripheral angiogenesis [57, 58]. However, the local delivery of PEDF into the affected eye of PDR or AMD patients to bolster the declining levels of PEDF in ocular tissues may result in the inhibition of unwanted vessel growth and potentially overcome unwanted side effects.

In theory, this approach may hold up for ocular neovascular diseases including PDR and AMD, and also serve as an adequate means to combat the activity of pro-angiogenic stimuli, such as VEGF.

## 6.3 Platelet-Derived Growth Factor Inhibitors

Platelet-derived growth factor (PDGF) is a potent mitogen known to be active on several cell types, in particular fibroblasts and vascular smooth muscle cells [59]. This growth factor is involved in enhancing vascular growth by promoting migratory and proliferative responses of endothelial cells as well as recruitment of

pericytes [60]. Four PDGF ligands namely A, B, C and D make up the PDGF family [61]. The aforementioned polypeptide chains which function as homodimers (PDGF-AA, BB, CC and DD) and heterodimers (PDGF-AB) recognize and bind to tyrosine kinase receptors PDGFRα and PDGFRβ [61]. Several studies have documented the role of PDGF in retinal NV. According to Seo et al., PDGF expression specific to photoreceptor results in severe retinal NV and retinal detachment [62]. Supportive evidence by Freyberger and colleagues also reveal increased PDGF levels in vitreous fluid of PDR patients [63]. Thus, the inhibition of PDGF remains an attractive option to treat ocular NV.

From a therapeutic standpoint, the antagonism of PDGF by a designed Ankyrin repeat protein (DARPin) which selectively binds to PDGF-BB has been shown to suppress retinal angiogenesis [64]. In this study, intraperitoneal injection (10 mg/kg) or intraocular injection (1.85 μg) of the anti-PDGF-BB DARPin significantly reduced subretinal and retinal NV in mouse laser-induced CNV and OIR, respectively. Furthermore, E10030 (Fovista – Ophthotech, New York, USA), an anti-PDGF pegylated aptamer, is in advanced stages of clinical trial for treatment of wet-AMD. Following the successful completion of a phase I safety and tolerability study which recorded no dose-related toxicities of E10030 in combination with ranibizumab in NVAMP subjects, data from the phase II study reported a similar favorable safety and efficacy profile in wet AMD participants [65]. In this study, E10030 in combination with anti-VEGF demonstrated statistically and clinically significant superiority in visual acuity gain compared to ranibizumab alone. Taking together these promising findings, a phase III study which will assess the safety and efficacy of E10031 in combination with anti-VEGF drugs compared to anti-VEGF alone has been initiated (Phase 3).

## *6.4 Integrin Receptor Blocker*

Integrins, which are a group of heterodimeric transmembrane proteins expressed by endothelial cells, are composed of α and β subunits and orchestrate the attachment between a cell and its surrounding extracellular matrix components including fibronectin, laminin, collagen, thrombospondin and fibrinogen [66]. The role of specific integrins $\alpha_v\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ has been reported in AMD [28]. In particular, integrin $\alpha_v\beta_3$ is known to be highly expressed within the endothelial cells of developing retinal blood vessels of DR patients and conversely, choroidal and retinal NV can be suppressed by $\alpha_v\beta_3$ antagonists [28], thus indicating that integrin $\alpha_v$ is a promising therapeutic target to treat ocular NV. Additional evidence has emerged from the use of a potent $\alpha_v$ integrin antagonist, JNJ-26076713, in rodent models of ocular vasculopathy [67]. Oral administration of this peptide which targets both $\alpha_v\beta_3$ and $\alpha_v\beta_5$, significantly attenuates retinal NV and reduces retinal vascular permeability in mouse OIR and diabetic rats, respectively [67].

Currently in clinical trial are two $\alpha_v\beta_1$ integrin antagonists, JSM6427 and Volociximab, for the treatment of AMD. Reports from Phase 1 clinical trial reveal JSM6427 to increase mean best corrected visual acuity in patients with exudative

AMD (https://clinicaltrials.gov/ct2/show/record/NCT00536016). Interestingly, $\alpha_v$ integrin mediates its effect in association with other pro-angiogenic factors, in particular VEGF. In line with this, it may appear prudent to develop combination therapy including $\alpha_v$ integrin antagonists and VEGF inhibitors. For example, a Phase 1 clinical trial assessing the safety and efficacy of intravitreal Volociximab in combination with ranibizumab for treatment of neovascular AMD showed to improve visual acuity in human subjects (https://clinicaltrials.gov/ct2/show/NCT00782093). Taken together, the potential of integrin antagonists may be positive indication for therapeutic intervention for ocular NV.

## *6.5 Thrombospondin-1 (TSP)*

Thrombospondin-1 (TSP1), a large extracellular glycoprotein, is a member of the TSP gene family typically secreted by RPE and vascular endothelial cells [68, 69]. TSP1 is widely known to orchestrate a wide array of cellular processes including cell migration, regulation of TGF-β during inflammation, wound healing and angiogenesis. TSP1 has been shown to be a major mediator of ocular homeostasis and congruently, retinal vascular development and NV are mitigated by increased levels of TSP1 [70]. The observations of low levels of TSP1 in choriocapillaries of AMD patients, and vitreous of DR patients also highlight the significant role that TSP1 plays in overall retinal vascular homeostasis [71, 72]. Recently, Wang and colleagues investigated the impact of TSP1 deficiency in a mouse model of CNV and the antiangiogenic influence of TSP1 peptide agonist. Here, it was evident that TSP1-deficient mice developed significantly larger areas of CNV compared to WT animals. Furthermore, this effect was shown to be reversed in TSP1-deficient mice following treatment with TSP1 mimetic peptide but to a greater extent in WT mice [73]. The archetypal phenomenon of AMD and DR is the display of both angiogenesis and inflammation in an exacerbated manner. Thus, TSP1 has also been reported to exert anti-inflammatory activity in the eye via the upregulation of TGFβ in RPE cells [74]. Altogether, these findings suggest that TSP1 plays a key role in the progression of CNV in AMD and that its modulation through TSP1 mimetic peptides could be perhaps complement current gold standard therapies for ocular NV diseases.

## *6.6 Peroxisome Proliferator-Activated Receptor Alpha (PPARα) Agonist*

Peroxisome proliferator-activated receptor-alpha (PPARα), a member of the nuclear receptor superfamily, is a ligand-activated transcription factor expressed in several tissues including the liver, intestine, kidney and skeletal muscle [75]. In association with its role in modulating lipid and glucose metabolism, this transcription factor

has also been reported to have anti-inflammatory and anti-angiogenic activities [76, 77]. The activation of PPARα is initiated via the binding of endogenous or synthetic ligands such as fatty acids or fibrate. Compelling evidence for therapeutic effects of PPARα agonist in retinal vascular leakage and NV is obtained from two large clinical trials; the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) and the Action to Control Cardiovascular Risk in Diabetes (ACCORD) which revealed preventive effects of Fenofibrate (PPARα agonist) in diabetes-related microvascular complications including DR in type 2 diabetes [78, 79].

In light of this, our group first demonstrated that fenofibrate significantly inhibits hallmarks of PDR and DME in rodent DR models [77]. Here, oral administration of fenofibrate suppressed retinal vascular leakage, leukostasis and levels of pro-inflammatory factors in STZ-diabetic rats and Akita mice. Furthermore, in STZ-diabetic rats and a separate OIR model of ischemic retinopathy, intravitreal injection of fenofibrate also attenuated retinal inflammation/hyperpermeability and retinal NV, respectively. Therefore, this indicates that the protective effect of fenofibrate on retinal inflammation and angiogenesis are independent of its systemic effect, and instead may be attributed to a direct ocular effect. Moreover, compared with current anti-VEGF drugs which are administered via invasive intraocular injections, the robust ocular effects of fenofibrate on DR and DME achieved by oral delivery along with its distinct pharmacokinetic behavior make PPARα agonists highly advantageous for the treatment of ocular NV diseases.

## *6.7 Wnt Pathway Blocker*

Wnts are a family of secreted cysteine-rich glycoproteins which regulate gene expression via both canonical and non-canonical Wnt signaling pathways. Of the two distinct cascades of Wnt signaling, the former has been reported to play significant roles in vascular development and angiogenesis [80]. Typically within the canonical pathway, the binding of Wnt ligands to the co-receptor complex of frizzled (Fz) receptors and low-density lipoprotein receptor-related protein 5 or 6 (LRP5/6), induces the subsequent phosphorylation and activation of downstream kinase nodes, leading to transcription of Wnt target genes such as VEGF, PDGF and TNF-α [81]. As previously reported by our group, retinal levels of total β-catenin, a key signaling factor of the canonical Wnt pathway, is significantly more abundant in patients with NPDR [82]. Additional confirmation of Wnt pathway activation in pathologic retina stemmed from data which also showed increased retinal levels of β-catenin and LRP5/6 co-receptors in several rodent models of retinal NV [82].

With this, it would appear prudent to inhibit the Wnt signaling pathway as a therapeutic strategy to treat ocular NV. In light of this, our group has convincingly shown the anti-inflammatory and anti-angiogenic activities of DKK1, a specific inhibitor of the Wnt pathway. Intraocular injection of this peptide decreased retinal levels of inflammatory marker, ICAM-1, and retinal vascular leakage in STZ-diabetic rats. In the same study, local injection of DKK1 into the vitreous of OIR

rats also appeared to reduce neovascular areas and tufts, as well as VEGF levels in rat retina, thereby demonstrating anti-angiogenic efficacy *in vivo* [82]. In a separate study, we also demonstrated the inhibitory effect of a monoclonal antibody (Mab) specific for the E1E2 domain of LRP6, Mab2F1, on canonical Wnt signaling and its therapeutic potential for DR [83]. In summary, Mab2F1 blocks the accumulation of β-catenin and overexpression of angiogenic/inflammatory factors in retinal cells. *In vivo* studies also reveal its anti-angiogenic and anti-permeability effects in OIR rats and late stages of STZ-induced diabetic rats [83]. Altogether, these studies showcase the therapeutic and beneficial effects of canonical Wnt signaling pathway inhibitors in ocular NV.

## *6.8 Corticosteroid Implants*

As noted, inflammation is a common pathological feature in PDR and DME. In light of this, corticosteroids have been noted to exert anti-inflammatory activity by blocking macrophage release of angiogenic factors and suppressing ICAM-1 expression, thereby stabilizing the BRB through increased tight junction proteins [84]. Three sustained-release corticosteroid implants currently in the development for treatment of DME include Ozurdex (Allergan), Iluvien (Alimera Science) and Retisert [85]. Ozurdex is a tiny biodegradable implant that slowly releases 0.7 mg dexamethasone into the vitreous and has been approved by the FDA for the treatment of DME secondary to BRVO or CRVO [86]. Both Iluvien (a nonbiodegradable polymer) and Retisert (a nonbiodegradable implant) release 0.19 mg and 0.59 mg fluocinolone acetonide into the vitreous, respectively [85]. The latter, which is typically inserted intravitreally, releases active steroid and has been approved in some European countries to treat chronic DME but not in the United States. Moreover, clinical efficacy studies in the United States have reported that Iluvien significantly reduced foveal thickness for up to 36 months [87].

## *6.9 Complement Cascade Inhibitors*

Typically, the complement system contributes to innate immunity and mediates the inflammatory responses seen in physiological and pathological conditions. There are several elegant studies implicating the link between ocular NV and the complement system as shown by increased levels of plasma C3adesArg in NVAMD subjects; and deposits of complement C5b-9 complexes in choriocapillaries of DN subjects [88, 89]. Complement targeted drug molecules are recognized as a promising therapeutic strategy for ocular NV diseases. A number of these compounds are currently in early stages of clinical trials. For example, POT-4/Compstatin (Potentia Pharmaceuticals/Alcon) is a “gel-like” synthetic peptide which binds and inhibits the cleavage of complement component 3 (C3) to its active form C3a and C3b [90].

Intravitreal injection of compstatin has been shown to suppress drusen formation in cynomolgus monkeys, primate model with early-onset macular degeneration [91]. Successful phase 1 safety and efficacy studies demonstrate therapeutic efficacy in AMD patients with subfoveal CNV (NCT00473928. http://www.clinicaltrials.gov/ct2/show/NCT00473928) and phase 2 clinical studies is in the pipeline to test efficacy of intravitreal POT-4 in neovascular AMD.

ARC1905 (Optotech Corporation) is a pegylated aptamer designed to target and prevent the cleavage of C5 into its active C5a and C5b forms [92]. A phase 1 clinical trial assessed the safety and tolerability of this anti-C5 aptamer in combination with anti-VEGF, Lucentis, in patients with wet AMD (NCT00709527. https://clinicaltrials.gov/ct2/show/NCT00709527)

### *6.10 Other Small Molecule Inhibitors*

Activation of cysteinyl leukotriene (CysLT) receptors, which are expressed in several tissues, mediates increased vascular permeability and ischemic retinopathy [93]. Of recent, Kennedy et al. identified an inhibitor of CysLT1 and CysLT2 receptor (CysLT1/2R), quininib, which demonstrated significant anti-angiogenic activity *in vitro* in EC tubular network assay, *ex vivo* in a mouse aortic ring assay and in zebrafish developmental angiogenesis assays. This CysLT1/2R antagonist was also reported to be safe and effective on preventing retinal NV in mouse OIR model when injected intravitreally [94]. Furthermore, to enhance its ocular release for up to 4 weeks, quininib was formulated into hyaluronan (HA) microneedles which showed to maintain its ocular anti-angiogenic and safety profile. In addition, intravitreal quininib-HA also attenuated CysLT-induced retinal vascular permeability in rats [95].

The phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling pathway has also been reported to be an alternative or adjunct target to treat ocular NV [96, 97]. Individual or combinations of PI3K/Akt/mTOR inhibitors including LY294002, NVP-BEZ235, PI-103 and rapamycin (Sirolimus) display significant anti-angiogenic effect in developmental and pathological angiogenesis in zebrafish and mouse retina [96, 98–100]. In particular, the development of sirolmus has transitioned up to Phase II clinical testing for treatment of AMD and DME (NCT01445548, http://www.clinicaltrials.gov/ct2/show/NCT01445548).

## 7 Conclusion and Perspective

Significant advances have been made towards understanding the molecular mechanisms regulating physiological and pathological angiogenesis. This progress has led to a comprehensive understanding of ocular NV which has yielded the discovery of current drug molecules to treat ocular NV-related diseases. The most common of these therapeutics are FDA approved anti-VEGF's such as ranibizumab and

aflibercept, and the off-label bevacizumab. It must be ceded that the future development of anti-VEGF therapies to treat ocular NV is undergoing a paradigm shift as a result of their limited therapeutic efficacies, high clinical costs, invasive intravitreal injections and unwanted side effects. Furthermore, ongoing research in the field of ocular anti-angiogenic therapy has offered what may prove to be alternative ways of treating NV-related blindness. A vast majority of the emerging drug therapies include, but not limited to those highlighted in this review. Among these, the PPARα agonist, fenofibrate, seem to be the most promising drug molecule for the prevention of ocular NV. Fenofibrate boasts high anti-angiogenic efficacy in animal models which recapitulate many of the clinical manifestations of CNV and retinal NV in humans. Moreover, the biopharmaceutical characteristic of the fenofibrate enables it to be delivered orally or as microparticles facilitating its sustained release over extended periods. In conclusion, indeed great strides have been made in the identification of novel therapies for the treatment of retinal NV, and the discovery of these new drug targets brings us one step closer toward the goal of delivering innovative therapies that are safe and more efficacious for patients affected by ocular NV.

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