Published in final edited form as:
J Genet. 2009 December ; 88(4): 495–515.

Mediators of ocular angiogenesis

Yureeda Qazi, **Surekha Maddula**, and **Balamurali K. Ambati***
Department of Ophthalmology, John Moran Eye Center, University of Utah, 65 Mario Capecchi Drive, Salt Lake City, UT-84132, USA

Abstract

Angiogenesis is the formation of new blood vessels from pre-existing vasculature. Pathologic angiogenesis in the eye can lead to severe visual impairment. In our review, we discuss the roles of both pro-angiogenic and anti-angiogenic molecular players in corneal angiogenesis, proliferative diabetic retinopathy, exudative macular degeneration and retinopathy of prematurity, highlighting novel targets that have emerged over the past decade.

Keywords

ocular angiogenesis; corneal neovascularization; retinal neovascularization; diabetic retinopathy; age-related macular degeneration; retinopathy of prematurity; VEGF; PEDF; Flt-1; Flk-1; endostatin; angiopoietin; erythropoietin; Tie2; inflammation; complement; gene therapy; TLR-3; Robo4

Introduction

Neovascularization (NV) is the formation of new blood vessels in previously avascular tissues by way of vasculogenesis and angiogenesis. Vasculogenesis is the formation of new blood vessels from bone marrow-derived angioblasts and is usually seen during embryogenesis. On the other hand, angiogenesis is the formation of new blood vessels from preexisting vasculature (Beck and D'Amore 1997; Isner and Asahara 1999) with an established role in tumour metastasis, corneal and retinal neovascular disorders (Folkman 1995).

Angiogenesis is the result of a complex interplay between growth factors, vascular endothelial cells, extracellular matrix molecules, chemokines and cell signalling molecules. It involves vascular endothelial cell activation, proteolytic endothelial basement membrane degradation, extracellular matrix degradation, endothelial cell migration, vascular proliferation, formation of tight junctions, recruitment of pericytes, and deposition of new basement membrane, closing off the newly formed arteriovenous collateral vessels (Folkman 1971; Yancopoulos *et al*. 2000; Carmeliet 2003).

Ocular angiogenesis can lead to irreversible visual impairment whether it is by opacification of the cornea or permanent deleterious changes to the neuronal architecture of the retina. This necessitates early and aggressive management of ocular neovascular conditions, possibly best done by targeting multiple putative factors. In our review, we explore these mediators of ocular angiogenesis and their roles in corneal neovascularization (KNV),

*For correspondence. bala.ambati@utah.edu.

proliferative diabetic retinopathy (PDR), exudative age-related macular degeneration (wet AMD) and retinopathy of prematurity (ROP).

Corneal neovascularization

Ocular surface disease, especially those leading to KNV poses a serious public health concern with considerable morbidity. The incidence of KNV in US stands at a grand 1.4 million with 4% of the population suffering from the condition (Lee *et al*. 1998). The dreaded complications of KNV include corneal oedema, lipid deposition, scarring and reduced chances of successful corneal grafts. Thirty per cent of vascularized corneas face the risk of graft failure following penetrating keratoplasty, making it imperative to identify the molecular mechanisms that may be targeted to prevent or retard its progression (Cursiefen *et al*. 1998).

The cornea bears an ‘angiogenic privilege’ and is avascular allowing maximal entry of incident light. This angiogenic privilege is maintained by a fine balance between anti-angiogenic factors and angiogenic factors in the cornea (Hanahan and Folkman 1996). Insults of chemical, mechanical, degenerative or infectious nature can trigger inflammatory and immune-mediated pathways which upregulate expression of VEGF (vascular endothelial growth factor), the key player in KNV, and its signalling cascades. Table 1 lists diseases which are associated with KNV.

Blood supply to the cornea originates from the ophthalmic artery which branches into the ciliary arteries and these arteries further divide to form the pericorneal limbal plexus. In corneal angiogenesis, neovessels arise from this pericorneal plexus (Burger *et al*. 1985; Yaylali *et al*. 1998) and sprout into the stroma. Depending on the underlying pathology, these blood vessels can either grow into the stroma in conditions such as viral stromal keratitis or form a vascular pannus which is more commonly seen in ocular surface disorders (Chang *et al*. 2001). Initial events in KNV as studied in rat corneas following chemical cautery involve vasodilation of the limbal vessels and leucocytosis. By 27 h, vascular buds emerge from the pericorneal venules and capillaries which lengthen, multiply and anastomose to form a network of blood vessels by day three. These vessels reach the site of injury by day seven and redundant vessels regress by day 14 leaving large tortuous vessels that merge with a pericorneal artery or vein (Burger *et al*. 1983). In human corneas, KNV commonly extends into the upper and middle third of the stroma (Cursiefen *et al*. 1998).

Several factors are known to play a role in KNV, among which we shall briefly discuss VEGF, basic fibroblast growth factor (bFGF), matrix metalloproteinases (MMPs), angiostatin, endostatin, pigment epithelium derived factor (PEDF) and review novel therapeutic targets uncovered in the past decade. A comprehensive list of the mediators of KNV is summarized in table 2.

Vascular endothelial growth factor

The VEGF family forms a part of the platelet-derived growth factor (PDGF) supergene family members of the VEGF family comprise VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and PlGF (placental growth factor). These cytokines bind to cell-surface receptors that belong to the family of tyrosine-kinase receptors (Shibuya and Claesson-Welsh 2006). VEGF-A has been studied extensively and plays a critical role in both vasculogenesis and angiogenesis (Hiratsuka *et al*. 2005; Sakurai *et al*. 2005; Takahashi and Shibuya 2005). VEGF is a secreted peptide found extensively in the epithelium of vascularized corneas secondary to inflammation (Kvanta 2006) and is a potent stimulator of hypoxia-induced corneal inflammation and angiogenesis (Singh *et al*. 2007). When the inflammatory cascade is interrupted preventing chemotaxis and endothelial migration, it is seen to remarkably

inhibit KNV as observed in CCR2 and CCR5 genetic ablative murine models (Ambati *et al*. 2003a,b). Alternative splicing of the VEGF gene yields five isoforms, VEGF115, VEGF121, VEGF165, VEGF189 and VEGF206 (Sugihara *et al*. 1998). VEGF is secreted by macrophages, T cells, retinal pigment epithelial (RPE) cells, astrocytes, pericytes and smooth muscle cells in response to hypoxic and inflammatory stimuli. VEGF binds to tyrosine-kinase receptors, VEGFR1 (Flt-1) and VEGFR2 (Flk-1), and also the neuropilins which lack tyrosine-kinase activity. VEGF signalling is modulated by angiopoietins that bind to Tie-2 receptors (Campochiaro 2004). Soluble Tie-2 receptors curb and regress KNV independent of VEGF in murine models of mechanical-alkali injury-induced KNV (Singh *et al*. 2005b). The avascular cornea owes it transparency to soluble VEGFR-1 (sFlt-1) that acts as a decoy receptor for VEGF-A, a potent stimulator of KNV (Ambati *et al*. 2006). Gene therapy with VEGFR-1 is anti-angiogenic when injected intracorneally in murine models of KNV (Singh *et al*. 2005a, 2006; Jani *et al*. 2007). Based on this evidence, using gene therapy to target specific receptors and pro-angiogenic molecules holds promise (Campochiaro 2006).

Fibroblast growth factor

Basic FGF (bFGF) belongs to the fibroblast growth family that encompasses 23 structurally related heparin-binding angiogenic peptides (Shing *et al*. 1984, 1985; Itoh and Ornitz 2004). FGFs are pleiotropic factors that act on various cells including endothelial cells. They interact with heparin-sulphate proteoglycans (HSPGs) and FGF receptors (FGFR-1, FGFR-2, FGFR-3, FGFR-4) like VEGF receptors bear tyrosine-kinase activity. It is perhaps their interaction with HSPGs that makes extracellular matrix (ECM) a key player in the regulation of angiogenesis. FGFs bind to their receptors found on target cells (Presta *et al*. 2005). FGF-1 and FGF-2 have been investigated extensively in noninflammatory KNV. FGF-1 is expressed in the normal corneal epithelium whereas FGF-2 is overexpressed after injury (Soubrane *et al*. 1990). Upon binding with their angiogenic ligands (FGF-1, FGF-2 and FGF-4), FGFRs are autophosphorylated leading to activation of several intracellular signalling pathways leading to the recruitment of Shc, FRS2 and Crk adaptor molecules (Cross and Claesson-Welsh 2001). Induction of endothelial cell proliferation by FGF leading to angiogenesis involves not only the activation of the mitogen-activated protein (MAP) kinase pathway, but also sustained activation of protein kinase C (PKC) (Presta *et al*. 1991). The angiogenic FGFs further promote angiogenesis by causing ECM degradation. FGF-1, FGF-2 and FGF-4 upregulate urokinase-type plasminogen activator (uPA) and MMP production in endothelial cells allowing localized proteolytic digestion at the vascular migration front (Mignatti and Rifkin 2000). However, this process is kept in check by the induction of plasminogen activator inhibitor (PAI)-1 providing fine control of the enzymatic ECM degradation (Kaneko *et al*. 2002).

Once the path for cell migration has been carved, FGF-1, FGF-2, FGF8b isoform and FGF-10 promote chemotaxis in endothelial cells (Stokes *et al*. 1990; Mattila *et al*. 2001). Endothelial cell migration also requires activation of the MAPK pathway (Shono *et al*. 2001). Cell migration and proliferation is carried out by FGF-2 regulated expression of integrins (alphavbeta3) and cadherins (Klein *et al*. 1993; Sepp *et al*. 1994; Underwood *et al*. 2002) with eventual maturation of the new vessels by inducing endothelial cell production of ECM components (Gerritsen *et al*. 2003). FGF-2 and VEGF/VEGFR systemsmaintain distinct biological roles but also work in concert to achieve angiogenesis (Presta *et al*. 2005).

Angiostatin—A 38-kDa proteolytic fragment of plasminogen is a robust anti-angiogenic factor (O'Reilly *et al*. 1994a,b). The cornea is a hub for angiostatin production as evident from its presence in the tear film of contact lens wearers (Sack *et al*. 1999), presence of plasminogen in the corneal epithelium (Twining *et al*. 1999) and detection of angiostatin-

producing MMPs (MMP-2, MMP-3, MMP-7, MMP-9 and MMP-12) . In murine models, angiostatin inhibits both bFGF-induced and injury-induced KNV (Ambati *et al*. 2002; Cao *et al*. 1999).

Endostatin—A 20-kDa proteolytic fragment of collagen XVIII bears anti-angiogenic properties (O'Reilly *et al*. 1997). It is generated by proteolytic cleavage of collagen XVIII by MMPs, cathepsin L and elastase. In the eye, collagen XVIII can be found in the cornea, retina and lens capsule (Lin *et al*. 2001; Ohlmann *et al*. 2005; Azar 2006). Endostatin inhibits both FGF- induced and VEGF-induced KNV (O'Reilly *et al*. 1997; Ferreras *et al*. 2000).

The PEDF is both anti-angiogenic and neurotrophic. It is found abundantly in RPE, iris and cornea (Ortego *et al*. 1996; Karakousis *et al*. 2001) and recently has also been detected in the tear fluid of healthy indivduals (Abdiu and Van Setten 2008). Since PEDF inhibits bFGF-induced KNV (Dawson *et al*. 1999) it may serve as an additional molecule sustaining the cornea's 'ocular privilege'.

Matrix metalloproteinases

MMPs are a group of zinc-binding proteolytic enzymes that engage in ECM remodelling and angiogenesis (Woessner 1994). The cornea expresses eight out of the 24 identified MMPs including collagenase I and III (MMP-1 and MMP-13), gelatinases A and B (MMP-2 and MMP-9), stromelysin (MMP-3), matrilysin (MMP-7) and membrane type-MMP (MMP-14) (Azar *et al*. 1996; Maeda *et al*. 1998; Ye and Azar 1998; Lu *et al*. 1999; Ye *et al*. 2000). The role of MMPs in KNV is ambiguous as the same molecule has the capacity to be both proangiogenic and antiangiogenic under different conditions (Itoh *et al*. 1998; O'Reilly *et al*. 1999).

Over the past decade, efforts to combat KNV have taken a turn towards gene therapy and nanotechnology. Of recent interest have been the Roundabout (Robo) receptor proteins which have an established role in neuronal guidance and are expressed in murine vascular endothelial cells (Robo4) during embryogenesis (Park *et al*. 2003). Robo4 is unique in that it is restricted to endothelial cells, especially at sites of angiogenesis. The soluble extracellular domain of Robo4 receptor (Robo4Fc) inhibits murine VEGF-induced and bFGF-induced endothelial cell migration (Suchting *et al*. 2005). Robo4 is an attractive candidate for gene therapy in KNV as latest evidence supports its antiangiogenic activities which have been established *in vitro* and in mouse models of retinal and choroidal vascular disease by inhibiting VEGF-165-induced vascular leakage and angiogenesis (Jones *et al*. 2008).

Proliferative diabetic retinopathy

Diabetic retinopathy (DR) is the leading cause of irreversible blindness in Americans of working age and the third leading cause of all blindness in US (Morello 2007). With the increasing prevalence of diabetes mellitus, DR looks set to pose a significant economic and quality of life burden among the US population (Paulus and Gariano 2009). The earliest histological features of diabetic retinopathy include capillary basement membrane thickening, loss of pericytes and loss of endothelial cells. At advanced stages, neovascularization occurs as part of proliferative DR (PDR). The breakdown of the blood retinal barrier and the consequent vascular leakage and thickening of retina are the main events involved in the pathogenesis of PDR.

Many biochemical mechanisms have been proposed to explain the development and progression of DR. Tight control of both blood glucose levels and hypertension are essential to prevent or arrest progression of the disease. Chronic hyperglycaemia leads to oxidative

injury, microthrombi formation, cell adhesion, molecule activation, leukostasis and cytokine activation. Next, ischemia leads to an overexpression of growth factors and cytokines. These factors include VEGF, insulin-like growth factor-1 (IGF-1), angiopoetin-1 and angiopoetin-2 (Ang-1/-2), stromal-derived factor-1, fibroblast growth factor-2 (FGF-2) and tumour necrosis factor (TNF). These growth factors act synergystically to mediate the steps of angiogenesis, including protease production, endothelial cell proliferation, migration and tube formation (Grant *et al*. 2004). Intraocular anti-angiogenic factors include PEDF, thrombospondin (TSP), TGF-B and somatostatin whose levels are reduced in the PDR environment. Ultimately, it is the balance between proangiogenic and anti-angiogenic factors that determines the development and progression of PDR (Simo *et al*. 2006). Because of the complex interplay among these factors, targeting a single growth factor is unlikely to result in therapeutic inhibition of angiogenesis.

Herein we examine the molecular mechanisms and genetic associations that contribute to neovascularization in the pathogenesis of PDR.

Angiogenesis

The agents involved in the development of diabetic retinopathy are diverse. In the setting of hyperglycaemia and retinal hypoxia, a number of vasoactive factors may interact to promote pathology in a variety of cell types including the microvasculature, neurons and glia. In addition to hyperglycemia, overproduction of reactive O_2 species by mitochondria, advanced glycation end products (AGEs), hexosamines and increased polyol metabolism of glucose leads to altered signalling of pathways involving protein kinase C, nuclear factor kappa-B (NFK-B) and MAP kinase. These changes damage retinal endothelial cells, pericytes, neurons, glia and pigment epithelial cells and recruit inflammatory cells which produce vasoactive compounds, growth factors, coagulation factors and adhesion molecules that eventually leading to angiogenesis and tissue remodelling (Pelikanova 2007).

Vascular endothelial growth factor

VEGF is universally accepted as the primary regulator of vessel patency in vascular networks throughout the body, including the retina. VEGF, a 40-kDa glycoprotein is pleiotropic and is involved in ROP, DR and AMD—the leading causes of irreversible visual loss in developed countries from infants to the elderly (Penn *et al*. 2008). Elevated levels of VEGF are associated with angiogenesis in PDR (Adamis *et al*. 1994).

Hyperglycemia leads to functionally and anatomically incompetent capillaries which leads to capillary nonperfusion, hypoxia and consequently induction of VEGF-A leading to angiogenesis (Crawford *et al*. 2009). VEGF induces complex formation of KDR-B3 integrin leading to phosphorylation of KDR. In mutant B3 integrin knock-in mice, VEGF-induced KDR phosphorylation was inhibited leading to incomplete formation of capillaries and *in vitro* inhibition of angiogenesis (Mahabeleshwar *et al*. 2006).

Hypoxia resulting from hyperglycemia-induced ischemia also promotes VEGF expression. *In vitro* studies with RPE cells under hypoxic conditions (1% of O_2) show increased expression of VEGF. Hsp90i and geldanamycin inhibit hypoxia-induced angiogenic response in RPE cells (Wu *et al*. 2007).

VEGF polymorphisms associated with PDR

A number of genetic polymorphisms within the VEGF gene have been associated with an increased risk of developing PDR. In Indian population, polymorphisms in the 5′UTR and promoter region of VEGF are thought to be a major genetic risk factor for DR (Suganthalakshmi *et al*. 2006). The AA genotype at −2578C/A polymorphism in VEGF

gene is associated with PDR, especially so in patients with diabetes for less than 15 years. While the −634C/G polymorphism in *VEGF* gene is not associated with PDR (Nakamura *et al*. 2009), it is associated with higher risk for DR in patients with microalbuminuria (Uthra *et al*. 2008). Many VEGF single nucleotide polymorphisms (SNPs) dictate the severity of PDR (Churchill *et al*. 2008).

The VEGF −460C polymorphism is a positive-independent-predictive factor for the development of proliferative diabetic retinopathy (Ray *et al*. 2004). Ray and colleagues used transfection studies to show that the VEGF −460 and VEGF +405 polymorphisms increased basal VEGF promoter activity by 71% compared with the wild-type sequence. Increased VEGF production from high-expressing haplotypes, including −460C, may promote neovascularization.

D allele I/D polymorphism in the promoter region of the *VEGF* gene is associated with retinopathy but not nephropathy in type 2 diabetes patients (Buraczynska *et al*. 2007). Multivariate logistic regression analysis showed that the D allele of the VEGF polymorphism is an independent risk factor of diabetic retinopathy after controlling other clinical variables.

VEGF and connective tissue growth factor

Connective tissue growth factor (CTGF) acts to promote fibroblast proliferation, migration, adhesion and extracellular matrix formation, and its overproduction is proposed to play a major role in pathways that lead to fibrosis (Moussad and Brigstock 2000). The ratio between CTGF and VEGF in the vitreous of PDR patients dictates the degree of fibrosis and angiogenesis. We know that the vitreous of PDR patients have elevated levels of both CTGF and VEGF. Raised CTGF levels are associated with VEGF and fibrosis, but only VEGF itself is responsible for NV in PDR. *In vitro*, CTGF-induced production of fibronectin and VEGF expression had no direct effects on vascular endothelial cells. CTGF may promote formation of proliferative membranes in PDR but not its cicatrization. It may be implicated indirectly in modulating VEGF expression but has no effects on retinal NV (Kita *et al*. 2007). Anti-VEGF therapy can temporarily tip the CTGF/VEGF ratio towards a profibrotic environment (Kuiper *et al*. 2008). CTGF induces growth of hyalocytes and bovine retinal pigment epithelial cells by activation of the MAPK pathway and ^{3}H-thymidine incorporation.

VEGF and inflammation

There is a distinct relationship between VEGF and the prostaglandin–cyclooxygenase system. Prostaglandins that mediate inflammation also influence retinal blood flow and have been found to be pro-angiogenic. Recent evidence suggests that cyclooxygenase-2 (COX-2) modulates angiogenesis by interacting with the VEGF system. Nitric oxide (NO) is a vasodilator and is implicated in VEGF mediated vascular permeability and angiogenesis. Emerging evidence indicates that COX-2 also interacts with NO and that these two systems have reciprocal effects on each other. There is little doubt that the interactions between these three vasoactive systems are complex and require further study in the context of retinal vascular permeability, angiogenesis and neurodegeneration (Wilkinson-Berka *et al*. 2004).

Monokines induced by IFN-y (Mig), like IL-1 and TNF-alpha, and VEGF levels were raised in vitreous of DR patients implicating the synergistic role of Mig with VEGF in the pathogenesis of retinal NV in DR (Wakabayashi *et al*. 2008).

Angiopoietins

Angiopoietin-1 and angiopoietin-2 interact with VEGF to promote angiogenesis in animal and *in vitro* models. Angiopoietin-2 levels were twice those of angiopoietin-1 in the vitreous of patients with nonPDR and clinically significant macular edema, implicating the role of Ang-2 in promoting VEGF-induced hyperpermeability that causes vascular leakage (Patel *et al*. 2005). Ang-1 is thought to act as an anti-permeability agent and is a natural antagonist of Ang-2. Increased plasma levels of VEGF and Ang-2, as well as lower soluble Tie-2, were found in diabetic patients. Together with increased VEGF, elevated Ang-2 levels offer a synergistic mechanism to stimulate vasoproliferation in PDR (Watanabe *et al*. 2005b). The highest VEGF and Ang-2 levels were seen among patients with preproliferative and proliferative retinopathy, but there was no relation of Tie-2 to the severity of retinopathy (Lip *et al*. 2004).

Erythropoietin

Erythropoietin (Epo), a stimulator of red blood cells, is also a promoter of vascular endothelial cell proliferation and angiogenesis (Bikfalvi and Han 1994). Both Epo and VEGF respond to hypoxia (Krantz 1991) leading to ischemia-induced angiogenesis. Epo and VEGF were both raised in the vitreous of patients with PDR and act independently of each other. Epo levels are higher than that of VEGF and its inhibition suppresses retinal NV both *in vivo* and *in vitro*. Suppression of Epo and VEGF leads to greater inhibition of retinal NV than when either is inhibited alone (Watanabe 2007). *In vitro* inhibition of Epo leads to attenuation of endothelial cell proliferation in PDR (Takagi *et al*. 2007). In murine models of oxygen-induced retinopathy, inhibition of Epo led to inhibition of retinal NV *in vivo* and inhibition of retinal endothelial cell proliferation *in vitro* (Watanabe *et al*. 2005a). Even though this evidence may tempt us to target Epo in the development of a retinal anti-angiogenic strategy, we must be cognizant of its neuroprotective effects on retinal cells (Becerra and Amaral 2002) and design with caution.

Anti-angiogenic factors

Given the prominent role of proangiogenic factors in the development of PDR, several anti-angiogenic factors have been identified as potential therapeutic agents. Prolactin-related vasoinhibin which has an antiangiogenic activity, is under-expressed in patients with DR (both proliferative and nonproliferative) compared to subjects without DR (0.041) (Triebel *et al*. 2009).

Certain dietary compounds (lutein, omega 3 fatty acids, eicosapentanoic acid and astaxanthin) have been thought to have antiangiogenic and anti-inflammatory effects, thus retarding CNV and DR (Ishida 2009) Puerarin, extracted from the Chinese herb *Puerariae lobata*, reduces expression of VEGF and HIF-1 in STZ-Wistar rats to reduce DR (Teng *et al*. 2009).

PEDF is an antioxidant glycoprotein, which is thought to play a significant antiangiogenic and hence protective role in the development of PDR. The aqueous of patients with PDR has significantly less PEDF than that of age-matched and sex-matched normoglycemic controls. Patients with PDR have low PEDF levels in the vitreous with elevated levels in the plasma (Ogata *et al*. 2007). Patients with PDR and CSME have high levels of vitreal VEGF and low levels of PEDF compared to nonproliferative DR (Patel *et al*. 2006; Yokoi *et al*. 2007). Lower levels of PEDF and higher levels of VEGF in the vitreous may be related to the angiogenesis in DR that leads to active PDR (Ogata *et al*. 2002). Animal models of DM-II as exemplified by the *db*/*db* mice also show increased levels of vitreal VEGF and low levels of PEDF at 18–20 weeks gestation consistent with early DR (Cohen *et al*. 2008). Antibodies to PEDF blocked vascular proliferation suggesting that levels of PEDF may determine

proliferative angiogenesis in PDR (Boehm *et al*. 2003). Vitreal levels of sFlt-1 and VEGF are significantly raised with corresponding decreased levels of PEDF in PDR patients versus patients with macular hole (Matsunaga *et al*. 2008). In addition, SNPs in the promoter region of PEDF, rs12150053 (TC and CC) and rs12948385 (GA and AA), are associated with DR. Increased susceptibility to DR in patients with these SNPs can be attributed to their increased interaction with the *VEGF* gene (Iizuka *et al*. 2007).

Inflammation

Inflammation plays an important role in the development of DR by promoting the invasion of pro-angiogenic inflammatory cells. Serum levels of patients with severe NPDR showed significantly elevated levels of RANTES/CCL-5 and SDF-1 alpha. CCL5 is chemotactic for T-cells, eosinophils and basophils, and plays an active role in recruiting leucocytes into inflammatory sites. FGF-2 promotes neovascularization by inducing pro-inflammatory mediators, recruiting monocytes/macrophages leading to NV (Presta *et al*. 2009). Diabetic retinas stain positive for ICAM-1/CD54, which mediates neutrophil adhesion, and the inner retina expressed MCP-1/CCL-2 and RANTES (Meleth *et al*. 2005). This indicates that in DR, a pro-inflammatory environment causes upregulation of vascular adhesion molecules that promote inflammatory cell infiltration and neovascularization.

The PDR retinal environment is characterized by upregulation of *iNOS, COX-2, ICAM-1, caspase 1, VEGF, NFK-B*, and increased production of NO, prostaglandin E2, IL-1beta, as well as increased permeability and leukostasis. Localized inflammation is responsible for capillary occlusion and degeneration leading to the ischemia-induced vasculogenesis, which results in PDR (Kern 2007). Increased leukocyte adhesion (via ICAM1-CD18) to retinal vascular endothelium with resulting endothelial damage, breakdown of the blood-retina barrier (BRB), capillary non-perfusion and ischemia contribute to neovascularization. Inhibition of integrin alpha-4, which forms a part of VLA-4 that binds to VCAM-1, decreases TNF-alpha, VEGF, NFK-b, reduces leukocyte adhesion and vascular leakage (Iliaki *et al*. 2009).

In addition to hyperglycemia, hypertension is one of the most common co-morbid conditions in diabetic patients and exacerbates inflammatory damage to the retina. STZ-induced diabetes in 4-weeks and 12-weeks old SHR and WKY rats revealed increased ED-1 + cells, ICAM-1 and VEGF in diabetic hypertensive rats implying earlier onset of retinal inflammation in diabetic retina as compared to their normotensive counterparts (Silva *et al*. 2007).

Cytokines produced by inflammatory cells play a central role in the pathogenesis of PDR by increasing promoting leucocyte-mediated damage to retinal vasculature (Adamis and Berman 2008). NADPH oxidase, produced by neutrophils, is associated with leukocyte adhesion and vascular leakage in DM and NV in OIR. NADPH oxidase is a mediator of DR possibly by reducing PPAR gamma and activating the NFK-B pathway (Tawfik *et al*. 2009). PPAR-gamma is reduced in DM, OIR and retinal inflammation, while NFK-B expression is activated in DM retina. *In vitro*, DPI, apocynin and SOD prevented suppression of PPAR-gamma in bovine retinal endothelial cells treated with high glucose.

In this environment, the balance is shifted in favour of MMPs and away from their inhibitors, tissue-inhibitor of matrix metalloproteinases (TIMPs). MMP-2 and MMP-9 actively degrade collagen IV, which is a major component of basement membranes, causing the ECM degradation needed for angiogenesis in DR. MMP-2 expression is much higher in patients who also have SNPs in the MMP-2 gene (Beranek *et al*. 2008). Such SNPs may predispose to PDR.

MMP-9 also degrades insulin and activates IL-8 thus recruiting inflammatory cells such as monocytes and neutrophils which release VEGF. MMP-9 induces inflammatory cell migration and degrades PEDF, which is an important anti-angiogenic protein in the eye (NadukKik and Hrabec 2008). Elevated levels of MMP-2 and MMP-9 contribute to the pathogenesis of PDR.

Chemokines and ECM molecules

Interleukins are primary mediators of inflammation, play an important role in DR pathogenesis and are prognostic markers of PDR disease severity. Patients with PDR have increased vitreal levels of IL-1 and TNF-alpha, which induce ICAM-1 expression (Demircan *et al*. 2006). Aqueous humour levels of IL-6 and VEGF correlate with respective levels in the vitreous and their concentrations increase with severity of disease (Funatsu *et al*. 2005). Early stage DR is associated with elevated levels of serum CD105 (which is thought to be involved in vascular remodelling) and vitreal VEGF which then decrease through the course of disease progression to severe PDR (Malik *et al*. 2005). IL-6 (T-cell activation), IL-8 (neutrophil chemotaxis), MCP-1 and VEGF levels are also significantly higher in the vitreous of patients with PDR (Murugeswari *et al*. 2008). IL-18, which induces macrophage activation via interferon-gamma, is raised in the sera of patients with DM2 and background DR (Skopinski *et al*. 2005). Thus, interleukins play an important role in mediating the inflammation and neovascularization in the development of PDR.

Growth factors and enzymes associated with DR

Insulin-like growth factor

Growth factors trigger the molecular events that induce retinal neovascularization. IGF-I is produced locally in the human eye by a variety of cells including RPE cells, retinal capillary pericytes, endothelial cells, Mueller cells and ganglion cells. Normoglycemic/ normoinsulinemic transgenic mice overexpressing IGF-1 in the retina developed most alterations seen in human diabetic eye disease (Ruberte *et al*. 2004). The two month-old transgenic mice showed loss of pericytes and thickening of basement membrane of retinal capillaries. In six months and older mice, venule dilatation, intraretinal microvascular abnormalities, and neovascularization of the retina and vitreous cavity were observed (Ruberte *et al*. 2004). Neovascularization was consistent with increased IGF-1 induced VEGF-expression in retinal glial cells (Ruberte *et al*. 2004).

In cultured human RPE cells, IGF-1 is thought to exert its effect by inducing a dose-dependent increase in IGF-1R phosphorylation and in VEGF mRNA levels. IGF-I also stimulates VEGF promoter activity *in vitro*, mainly via HIF-1alpha, and secondarily via NFK-B and AP-1 (Poulaki *et al*. 2004). In a south Indian cohort, a CA 18-repeat genotype in the promoter of IGF-1 is implicated in susceptibility to PDR and associated with clinical severity (Uthra *et al*. 2007).

Further evidence for the role of IGF-1 in PDR comes from the use of IGF-1 inhibitors. Somatostatin and octreotide, a somatostatin analogue, inhibited IGF-1 receptor (IGF-1R) phosphorylation and decreased VEGF production (Sall *et al*. 2004). Systemic inhibition of IGF-I signalling in a relevant animal model with a receptor-neutralizing antibody, or with inhibitors of PI-3 kinase (PI-3K), c-Jun kinase (JNK), or Akt, suppressed downstream signalling pathways, VEGF expression, ICAM-1 levels, leukostasis, and BRB breakdown. Intravitreous administration of IGF-I increased retinal Akt, JNK, HIF-1alpha, NFK-B, AP-1 activity, and VEGF levels. Therefore, understanding of the molecular pathways by which IGF-1 plays a role in PDR may lead to the development of specific therapies based on inhibition of either IGF-1 or its downstream actors (Poulaki *et al*. 2004).

Aldose reductase

Aldose reductase (AR) is responsible for the early events in the pathogenesis of diabetic retinopathy, including BRB breakdown, loss of pericytes, neuro-retinal apoptosis, glial reactivation, and neovascularization. In *db*/*db* mice with a null mutation for AR, retinal blood vessels were found to leak IgG suggesting that AR may contribute to BRB breakdown. AR deficiency also prevented diabetes-induced reduction of platelet/endothelial cell adhesion molecule-1 expression and increased expression of VEGF, which may have contributed to blood-retinal barrier breakdown. In addition, long-term diabetes-induced neuro-retinal stress and apoptosis and proliferation of blood vessels were less prominent in AR−/− *db*/*db* mice (Cheung *et al*. 2005).

The renin-angiotensin system (RAS)

Both human and rat retinas have angiotensin receptor (ATR) type-1 and ATR-2. In both human and rat models of DR and hypoxia-induced retinal angiogenesis the RAS is upregulated leading to the production of VEGF, PDGF and CTGF leading to microvascular complications, angiogenesis, cell proliferation and fibrosis (Wilkinson-Berka 2006).

The RAS exerts its effects by the generation of a family of bioactive angiotensin peptides among which angiotensin II (ANG II) and the ATR-1 and ATR-2 receptors are most well characterized (Wilkinson-Berka *et al*. 2004). Emerging evidence suggests that an ocular RAS is activated in DR and may contribute to progressive alterations to retinal cells such as pericytes, endothelial cells, neurons and glia. In the kallikrein-kinin system (KKS), bradykinin (BK) and kallidin and their carboxypeptidase metabolites, des-Arg(9)-BK and des-Arg(10)-kallidin are the effector peptides exerting their actions via BK type 1 (BK-B1) and BK type 2 (BK-B2) receptors. Both RAS and KKS damage the retinal vasculature and glia in DR via production of VEGF and CTGF (Wilkinson-Berka *et al*. 2004).

The RAS is also implicated in progression of DR via Ang II. Ang II induces VEGF, which leads to the loss of tight junction proteins causing a breach in the integrity of the BRB. Angiotensin receptor blockers that block Ang II receptors reduce VEGF production by retinal endothelial cells and promote the recovery of tight junction proteins thus preventing progression of DR in its early stages (Kim *et al*. 2009).

Important cross-talk exists between the RAS system, advanced glycation end products (AGEs) and their receptors (RAGE). AGEs act via RAGE to cause diabetic microvascular complications leading to PDR (Yamagishi *et al*. 2005). *CCN1*/*Cyr61* is a member of the cysteine-rich 61/connective tissue growth factor/nephroblastoma overexpressed (CCN) family of genes. It is a downstream effector of AGE in the diabetic retina and may work synergistically with VEGF to cause ocular angiogenesis and PDR in models of oxygen induced retinopathy (OIR) in mice and streptozotocin (STZ)-induced DM in rats. Levels of both *CCN1* mRNA and protein are raised in vitreous of STZ rats and PDR patients (non-diabetics) (Hughes *et al*. 2007). AGEs-RAGE-induced VEGF expression is thought to lead to neovascularization in PDR. Olmesartan, an angiotensin II type-1 receptor blocker, inhibited angiogenesis by inhibiting AGE-induced NFK-b promoter activity and consequently NFK-b—mediated RAGE expression (Yamagishi *et al*. 2008). AGEs also induce injury of retinal pericytes, which are protected by PEDF expression. Thus, a decrease in PEDF expression can amplify the effect of AGEs on RPE integrity leading to PDR (Chmielewska *et al*. 2008).

Retinopathy of prematurity

Retinopathy of prematurity (ROP), formerly known as retrolental fibroplasia, was first described in 1942 and is one of the leading causes of blindness in children (Terry 1942).

While we have ablative treatments today such as cryotherapy and lasers, it is important to understand the molecular pathogenesis as it may help us discover preventive methods. The main pathology in ROP is abnormal angiogenesis. In the normal-term baby, there is a vasculogenesis phase in which the blood vessels sprout off the hyaloid and create the vasculature reaching toward the retina until week 21 of gestation (Hughes *et al*. 2000). The second phase termed the angiogenic phase, overlaps with the first, beginning at 17 weeks and is driven by physiological hypoxia. The existing vasculature remodels and branches to create more vessels to meet the higher oxygen demands of the growing structures (Ashton *et al*. 1954; Hughes *et al*. 2000). ROP occurs in premature infants with disruption of this angiogenic phase. Premature neonates are exposed to higher oxygen levels in the early stages of retinal vascular development, eliminating physiological hypoxia, thus downregulating angiogenic factors that are necessary for the growth of the vasculature. This results in a vaso-obliterative phase and is seen in infants born prematurely at 30–32 weeks (Ashton *et al*. 1954; Madan and Penn 2003; Chen and Smith 2007). Since angiogenesis is interrupted, the retina becomes hypoxic, inducing the proliferation of new vessels between the vascularized and avascular retina; this is termed the vaso-proliferative phase of ROP (Ashton *et al*. 1954; Smith 2008). The pathological vasculature can cause fibrous scarring in the retina, vitreous and lens causing traction which can lead to retinal detachment and blindness (Chen and Smith 2007).

Oxygen and hypoxia inducible factor

As described earlier, after vasculogenesis, angiogenesis in the normal retina *in utero* is driven by hypoxia. This high oxygen demand is mostly due to rod development (Arden *et al*. 2005). The main pathology in ROP is that the baby is prematurely exposed to abnormal oxygen levels, which govern development. Hypoxia-inducible factor-1alpha (HIF), is a transcription factor that upregulates angiogenic factors under hypoxia. When the oxygen tension is normal, HIF is rapidly oxidized by hydroxylase enzymes, but when cells become hypoxic, HIF escapes degradation and accumulates, triggering the activation of a number of genes, like VEGF and EPO (Arjamaa and Nikinmaa 2006). This system is tightly regulated; even a mild change in regimen of the ROP induction model to 45 to 12.5% O_2 from the 40 to 15% O_2 model yielded 78% increase in release of VEGF. The release of VEGF is highly sensitive to the slightest change in oxygen levels and the amount of change in oxygen level (Werdich *et al*. 2004). Inhibiting prolyl hydroxylase, an inhibitor of HIF has been shown to reduce ROP in mice. This may be a possible therapeutic intervention by chemically stabilizing HIF and maintaining the conditions of ‘physiological hypoxia’ (Sears *et al*. 2008). One study showed that inducible nitric oxide (iNOS) may be the one mediating HIF activation via PI3K/Akt signalling pathway and may be an other avenue of intervention (He *et al*. 2007).

VEGF

VEGF is an important regulator of angiogenesis and plays a crucial role in both phases. Unlike other factors it is not constitutively produced but temporally and spatially induced during vasculogenesis (Murata *et al*. 1996). Normally during angiogenesis, VEGF levels should rise due to the elevation in HIF (Shweiki *et al*. 1992; Penn *et al*. 2008). However in phase 1 of ROP, these levels decrease within 6 h under normoxic or hyperoxic conditions (Pierce *et al*. 1995, 1996; Shweiki *et al*. 1992). VEGF is strictly under the control of oxygen and HIF; between 17% and 45% oxygen, the extent of vasculogenic cell division is inversely proportional to the level of oxygen (Chan-Ling *et al*. 1995). In ROP, VEGF is the ‘master switch’, its low levels decrease angiogenic signalling and allow the retraction of blood vessels by apoptosis of endothelial cells. Exogenous administration of VEGF and VEGFR-1 specific ligand can counteract this effect of hyperoxia in the first phase of ROP (Alon *et al*. 1995; Pierce *et al*. 1996; Shih *et al*. 2003; Wilkinson-Berka *et al*. 2004).

In stage two of ROP as the retina becomes hypoxic, VEGF levels rise within 6–12 h (Pierce *et al*. 1995). VEGF is produced by neighbouring astrocytes and affect endothelial cells in a paracrine fashion. These endothelial cells then exhibit high affinity receptors Flk-1 (also known as VEGFR2), which are specific for proliferative neovasculature (McLeod *et al*. 2002).

VEGF is a major target for therapeutic intervention in ROP. Thus far, VEGF inhibition via monoclonal antibodies and hyperoxia, and factors such as thrombospondin-1 and PDGF, have been used to decrease the proliferative angiogenesis in phase 2 of ROP (Pierce *et al*. 1996; Suzuma *et al*. 1999; Wilkinson-Berka *et al*. 2004). Several other inhibitors such as alpha versus beta integrins, alpha-defensins, nonpeptide antagonists and PEDF decrease VEGF mediated migration, EC permeability and proliferation and may have a potential therapeutic application (Hutchings *et al*. 2002; Economopoulou *et al*. 2005; Wilkinson-Berka *et al*. 2006). Eph receptor tyrosine kinase blockade has also become an area of interest as it inhibits new vessel formation without interrupting existing structure (Chen *et al*. 2006). Angiotensin 2 receptor blockade has also been explored as recent studies have shown the involvement of the RAS system in angiogenesis (Sarlos *et al*. 2003). More recently, gene therapy such as anti-VEGF SiRNA has been effective in reducing retinal neovascularisation (Jiang *et al*. 2009).

Erythropoietin

Erythropoietin (EPO) is a hormone that is initially secreted in the foetal liver and later by the kidney as an adult. Though EPO is known for its hematopoietic qualities, recently it has gained interest in its influence on angiogenesis after success in stroke therapy. Studies have shown that it effects endothelial cells and angiogenesis as much as VEGF but through an independent mechanism (Jaquet *et al*. 2002; Sato *et al*. 2009). EPO is part of normal eye development; expression of the protein and receptor can be found in the retina and its vasculature (Chen *et al*. 2008). While HIF levels stay the same during embryonic development, EPO serum levels rise about seven fold from 15–24 weeks of gestation. A recent study measuring mRNA and protein concentrations have shown that EPO levels are six times higher in the vitreous than in the plasma during gestation weeks 15–17. This difference decreases to two-fold by gestational age 21–24 weeks (Patel and Chan 2008). Like VEGF, EPO is also regulated by HIF or HIF-1alpha like factor (HLF), and its levels are reduced under hyperoxic conditions. In HLF deficient mice under OIR (oxygen induced retinopathy) showed no ROP and decreased levels of EPO (Morita *et al*. 2003).

Timing is critical in the role of EPO. Studies have shown that EPO levels are low during the vaso-obliterative phase. Administration of exogenous EPO during this phase inhibited retraction and stabilization of vessels due to proangiogenic bone marrow derived progenitor cells. It also has neuroprotective qualities such as resistance of hypoxia induced neuronal apoptosis, reduction of reactive oxidative stress, and inhibition of inflammatory chemokines (Chen *et al*. 2008; Wang *et al*. 2009).

However, in the vasoproliferative phase of ROP, exogenous EPO administration can be harmful. Retrospective studies have shown the incidence of retinopathy of prematurity increase when EPO was used to reduce blood transfusions in premature children (Brown *et al*. 2006; Suk *et al*. 2008). ROP occurs if given during phase two or around eight days after birth by augmenting pathological angiogenesis (Schneider *et al*. 2008). So far, interference technology has been successful in preliminary studies showing anti-EPO siRNA significantly reducing ROP in mice (Chen *et al*. 2009).

IGF-1

IGF-1 is a maternally derived factor that is provided by the placenta and amniotic fluid (Langford *et al*. 1998). In addition to post-menstrual age at birth and low birth weight, studies have shown that low IGF serum levels can be used as a screening factor for predicting ROP (Hellstrom *et al*. 2003; Villegas-Becerril *et al*. 2006). Further interrogation has revealed that these factors have a direct influence on angiogenesis. In a mouse model, GH inhibition decreased ischemia induced neovascularization which was recovered by exogenous administration of IGF-1 (Smith *et al*. 1997).

While VEGF is increased by HIF under hypoxia, IGF-1 is required for the angiogenesis to take place. *In vitro* studies have shown that low levels of IGF-I prevent VEGF-induced activation of protein kinase B (Akt), a kinase critical for endothelial cell survival (Hellstrom *et al*. 2001) It is thought that if IGF levels are insufficient at birth, the retina stays avascular and VEGF levels begin to accumulate until IGF levels reach a threshold, at which point the vasoproliferative phase begins. IGF-1 receptor regulation of VEGF action is mediated through control of VEGF activation of p44/42 mitogen-activated protein kinase (Smith *et al*. 1999). Increasing levels of IGF or exogenous administration of IGF to maintain high levels in a premature infant may be a potential avenue of therapy (Smith 2005; Vanhaesebrouck *et al*. 2009). Somatostatin, an inhibitor of growth hormone plays a role in regulation as its receptors (sst2) have also been identified on the RPE. Physiological amounts of somatostatin and its analogue octreotide inhibit VEGF and IGF-mediated neovascularization and may also be used as a potential therapy (Sall *et al*. 2004).

Omega-3 PUFAs

Like IGF, Omega-3 PUFAs (omega 3 polyunsaturated fatty acids) are maternally derived and given to the foetus in the third trimester, and this is deficient in premature children (Connor *et al*. 2007). Omega-3 PUFAs have shown to decrease avascular areas and increase vessel regrowth after injury. In addition, neuroprotectinD1, resolvinD1 and resolvinE1 are all derived from omega-3 PUFAs. These bioactive metabolites are neuroprotective in addition to reducing TNF alpha mediated angiogenesis. Neuroprotectin D1 also aids in brain cell survival and repair involving neurotrophic, anti-apoptotic and anti-inflammatory signalling. Lack of these properties may contribute to the susceptibility of ROP (Mukherjee *et al*. 2007; Lukiw and Bazan 2008).

Exudative age related macular degeneration

Age related macular degeneration (ARMD) is the major reason for blindness in the elderly population. It is a multifactorial disease that progresses from damage of the retinal pigment epithelium and Bruchs membrane, leading to the phenotypes of geographic atrophy and neovascularization. The latter, exudative type involves abnormal angiogenesis causing choroidal neovascularization under the retinal pigment epithelium which can ultimately lead to blindness. There are a number of factors we will discuss in this next section that mediate angiogenesis and help us gain a better understanding of the mechanisms and pathogenesis of wet ARMD.

VEGF

Originally known as vascular permeability factor and discovered in tumour cells, VEGF has become the main factor that mediates angiogenesis in ARMD. VEGF, a relative of the PDGF family is a secreted protein mitogen (Keck *et al*. 1989; Leung *et al*. 1989). It acts by promoting endothelial cell proliferation and survival, ocular inflammation and increasing permeability by creating fenestrations in post-capillary and muscular venules and capillaries (Roberts and Palade 1995). In addition, it is the major regulator of a number of downstream

factors. Despite its pathological role in ARMD, VEGF is a protein that is necessary for early life and normal eye development. Deficiency in the VEGF gene causes hypoplastic vasculature and death *in utero* (Carmeliet *et al*. 1996).

VEGF is constitutively produced, but in ARMD, its levels are elevated due to damage, ischemia and hypoxia. VEGF is produced at a much higher concentration basally near the VEGF receptors on the choriocapillaris under hypoxic conditions (Blaauwgeers *et al*. 1999).

While VEGF is the main regulator in ARMD, increased VEGF alone is not enough to cause subretinal neovascularization. A transgenic model of increased VEGF production by RPE and choroid showed that vasculature is increased but only within the choroid and none had invaded Bruch's membrane. Additional environmental insults and the balance of the angiogenic versus anti-angiogenic factors determine whether choroidal neovascularization will be present (Schwesinger *et al*. 2001).

Pigment epithelium derived factor

PEDF, initially known as a neurotrophic factor, has antiangiogenic properties that maintain the eye free of vascularization by reducing VEGF induced chemotactic endothelial cell migration and proliferation via apoptosis (Mori *et al*. 2001). While it is present in many of the ocular tissues constitutively, it is highly expressed in the RPE covering CNV at much higher levels when neovascularization first occurs (Ogata *et al*. 2002).

Most studies have demonstrated PEDFs inverse relationship to CNV, its downregulation the likely cause of CNV (Shi *et al*. 2004). A cadaver eye study measuring VEGF and PEDF showed significant decreased in PEDF in choroidal tissues with those with ARMD as compared to age matched normal (Bhutto *et al*. 2006). Aqueous humour of ARMD patients with active CNV exhibited angiogenic properties (Tong *et al*. 2006). PEDF levels measured in CNV/AMD vitreous was on average 2.8 ng/μL +/− 1.3 ng/μL compared to 16.4 ng/μL +/− 7.1 ng/μL in normal age matched vitreous. In addition, the vitreous of normal adults had no endothelial chemotactic properties and even resisted VEGF-induced migration (Holekamp *et al*. 2002). In the retina, the VEGF/PEDF ratio is 10:1 with neovascularization, and increases as vascularization progresses. This has been shown by measuring mRNA concentrations of the factors in the ARMD rat model as compared to age-matched controls (Gao *et al*. 2001; Renno *et al*. 2002). In late stages of ARMD, PEDF is directly related to the oxygen levels in the eye and is reduced in ischemic eyes or those under oxidative stress. (Dawson *et al*. 1999; OhnoMatsui *et al*. 2001).

VEGF and PEDF seem to regulate each other via feedback mechanisms in a tightly controlled manner. An examination of cultured endothelial cells (CEC) displayed how PEDF had no impact on normal cells while it inhibited the migratory effects of VEGF-induced endothelial cells (Wang *et al*. 2007). PEDF is upregulated by VEGF through VEGFR-1 in human RPE. It is mediated by two different pathways: intramembrane proteolysis of VEGF-1 and inhibition of VEGFR-1 phosphorylation by VEGF (OhnoMatsui *et al*. 2003).

PEDF decreases CNV, but only if administered exogenously in high amounts during initial vascularisation (Ohno-Matsui *et al*. 2001). Thus far, high doses of PEDF have been successful via adeno association vector in mice (Mori *et al*. 2002) and in pigs (Saishin *et al*. 2005). However this was contradicted when the various doses of administered PEDF were taken into account. While a low dose was inhibitory, high dose of PEDF augmented neovascularization with VEGF (Apte *et al*. 2004). Intriguingly, a PEDF knockout mouse model had an entirely normal ocular phenotype (Yoncopoulos G. and Wiegand S. PEDF-

knockout-mice have a normal ocular phenotype. A poster presentation at Association for Research in Vision Science and Opthalmology, Fort Louder date, May 2007).

There is contradictory evidence of the role of PEDF. We now know that it initially increases when neovascularization occurs. This is likely a negative feedback mechanism to the increased VEGF. Overall, it is the decrease in PEDF that probably makes one susceptible to ARMD. While exogenous PEDF had been shown to be successful in what..?, It seems that timing and dosage is crucial and it may turn out to be detrimental if used incorrectly. More recently, the role of genetics have also been explored in ARMD, and a recent study implicates the T allele of Met72Thr (T/C) of *PEDF* gene exon 3 to be linked with wet ARMD (Lin *et al*. 2008).

Fibroblastic growth factor

Similar to VEGF, fibroblastic growth factor (FGF) plays a crucial role in the development of the eye. Studies with mice deficient in FGF receptor 1 and FGF inhibition show that the choroid is thinned and the RPE and photoreceptor integrity is compromised in hemizygous mice. In homozygous deficient mice for FGF, eye growth is interrupted and there is severe degeneration (Rousseau *et al*. 2000). FGF is responsible for development of the eyes vascular plexus, regression of the hyaloid plexus and the induction of choroidal terminal branching during embryogenesis. VEGF(165) and FGF2 play a synergistic role, both significantly increase human macular endothelial cell proliferation and sprout formation (Browning *et al*. 2008).

Analysis of eyes with CNV versus normal ones via immunohistochemistry has shown a high presence of acidic fibroblastic growth factor (aFGF) and basic fibroblastic growth factor bFGF the latter being more common (Amin *et al*. 1994). The deeper choriocapillaris within the stroma exhibits aFGF wheareas the bFGF is the more common type in angiogenesis. Infusion of bFGF growth factor into mini pigs has demonstrated that bFGF has direct effect of choroidal neovascularization (Soubrane *et al*. 1994). Under normal conditions FGF-1 receptor mRNA is seen in the ganglion cells and the inner nuclear layer. However, during CNV, FGF production also appeared in the retinal pigment epithelial cells, in melanocytes of the choroid and in the choroid endothelial cells (Matsushima *et al*. 1996). Studies have also shown a role for FGF5 in ARMD, expressed in blood vessels and the surrounding extracellular matrix of the choroid (Kitaoka *et al*. 1997). Beta-FGF works via two pathways; via a calcium independent FGFR1 through PI 3-K, P70(S6K) and Akt to increased VEGF A from the RPE and a calcium dependant FGFR2 which is cascade of MEK1, ERK1/2 and P90(RSK). The inhibition of both ERK1/2 and PI 3-K activities suppresses bFGF-induced choroidal endothelial cells proliferation (Zubilewicz *et al*. 2001; Rosenthal *et al*. 2005).

In addition to FGF, other growth factors such as connective tissue growth factor (CTGF) and transforming growth factor (TGF) are elevated and colocalized in the RPE of retinas with CNV. These two factors, stimulate fibroblasts to produce VEGF along with extracellular matrix and promote angiogenesis (Nagineni *et al*. 2003; Watanabe *et al*. 2005c).

Angiopoietins

Like VEGF, angiopoietins are growth factors that are specific to endothelium. Angiopoietin-1 (Ang1) is a ligand for the Tie-2 receptor and an angiogenic factor that mediates communication between the endothelium and the ECM molecules. These molecules also affect late embyrogenesis along with FGF; overexpression of Ang1 allows higher branched vasculature (Suri *et al*. 1996, 1998). Angiopioetin 2 (Ang2), discovered later, is a natural antagonist of Ang1 and mediates inhibition of angiogenenesis during embryogenenesis and *in vivo*. Ang2 is found in areas undergoing vascular remodelling and

destabilizes existing blood vessels (Witzenbichler *et al*. 1998). Interestingly Ang2 only causes CNV under the influence of VEGF; in the absence of VEGF, Ang2 causes regression of vessels (Peters *et al*. 1998). VEGF increases endothelial cell proliferation of thin vessels that are weak and leaky. Working alone, Ang1 stabilizes vessels and counteracts their vascular permeability (Nambu *et al*. 2004). Together, these factors have an additive effect producing abundant stable vessels during neovascularization (Nambu *et al*. 2005). While Ang1 is technically angiogenic, in ARMD it appears to be protective rather than pathological allowing the reduction of microvascular leakage. Ang1 has also been found to inhibit the proinflammatory qualities of VEGF such as the upregulation of ECM molecules I-CAM, V-CAM and E-selectin to induce leukocytosis (Kim *et al*. 2001).

Several studies showed that Tie-2 signalling decreased choroidal neovascularization by adenovirus-mediated gene delivery of extracellular domain of the Tie-2 receptor (Hangai *et al*. 2001) and inhibitory molecules of Tie-2 receptors. This may be a new avenue of therapy via angiopoietin and the Tie-2 axis (Liu *et al*. 2008).

Nitric oxide

Through many studies, we have long known that NO is mediator of angiogenesis by way of VEGF (Papapetropoulos *et al*. 1997). Studies have demonstrated this by inhibiting NOS and measuring the amount of NO that was produced (Papa-petropoulos *et al*. 1997; Uhlmann *et al*. 2001). Interestingly, timing and duration of exposure determines the type of NOS produced; long term exposure of VEGF stimulates endogenous nitric oxide synthase (eNOS) while short term exposure promotes NO release through activationg of tyrosine and PI-3K kinases. The latter inducible form (iNOS) is the pathological NO mediated by cytokines in ARMD (Hattenbach *et al*. 2002). Flow cytometry studies show when iNOS is upregulated there is an increase in alpha versus beta(3) integrin expression on endothelial cells and macrophages indicating increased migration and adhesion (Lee *et al*. 2000; She *et al*. 2007).

While iNOS may be responsible for choroidal neovascularization in ARMD, it plays a different role in oxygen-induced ischemic retinopathy. In a comparison between iNOS deficient mice and eNOS deficient mice, VEGF stimulation significantly increased permeability in both wild type and *iNOS*(−/−) mice but not in *eNOS*(−/−) mice, suggesting that eNOS plays a predominant role in hypoxia-induced angiogenesis and vascular permeability (Fukumura *et al*. 2001). In the latter case, deficiency of eNOS was responsible for retinal neovascularization and iNOS was actually inhibitory (Ando *et al*. 2002).

More recent studies looking into the relationship between fatty acids and inflammation have shown that certain fats such as oleic acid, linoleic acid and linolenic acid increased the expression of *iNOS* and *COX-2* genes and the production of prostaglandin E2 in the RPE. Linoleic acid also induces *NF-kappaB* transcriptional activation which promotes inflammatory pathogenesis of ARMD. On the other hand, longer unsaturated fatty acids such as Lutein are protective and block *NF-KappaB* activation and reduce inflammatory factors in dose-dependent manner (Fang *et al*. 2009).

ECM molecules

Studying the basal lamina and linear deposits (BLD) can provide valuable insights about the key players in the pathogenesis of ARMD. Recent study of BLD in CNV eyes demonstrated VEGF, vitronectin, MMP-2, MMP-7, MMP-9, TIMP-3 and complement C3b and C5–9 complexes (Lommatzsch *et al*. 2008).

I-CAMs are adhesion molecules that promote VEGFs chemotactic ability. Its inhibition prevents permeability and leukostasis (Miyamoto *et al*. 2000). Studies of ICAM-1 and

leukocyte adhesion molecule CD 18 deficient mice show significantly less CNV than wild-type mice (Sakurai *et al*. 2003b).

MMPs are a family of zinc dependant endonucleases that are involved in degradation of the extracellular matrix. MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9 have been implicated in its relationship to wet ARMD. MMP-1 and MMP-3 are found only in Bruchs membrane where MMP-2 and MMP-9 were found in the choroid. Production of MMP-2 and MMP-9 by the RPE increases when stimulated by VEGF, fibronectin and TNF-alpha (Hoffmann *et al*. 2006). This suggests that some MMPs may be selective and can be part of the pathological breakdown and remodelling of the matrix during neovascularization in ARMD (Friedlander *et al*. 1995; Guo *et al*. 1999; Kadonosono *et al*. 1999). However, in experimentally induced CNV, only MMP-2 has been shown to be associated with ARMD. MMP-2 increases in macrophages and the RPE invading the choroid (Kvanta *et al*. 2000). It increases from day three and peaks at day five after the initiation of CNV (Berglin *et al*. 2003). Compared to age-matched control eyes, the amount of MMP-2s double in the RPE of ARMD patients (Plantner *et al*. 1998). In addition CNV was significantly reduced in genetically engineered MMP-2 deficient mice (Berglin *et al*. 2003).

Sorsby's fundus dystrophy is an inherited form of blindness which has extensive choroidal neovascularization due to a mutation in TIMP-3; leading to a premature onset of an ARMD-like phenotype (Anand-Apte *et al*. 1997). TIMPs are also a group of zinc-binding endopeptidases that counteract the actions of MMPs. TIMP-3 has been localized to to the q12.1–q 13.2 region of human chromosome 22 (Apte *et al*. 1994) TIMP-3 inhibits chemotaxis of vascular endothelial cells toward VEGF and bFGF, inhibits collagen gel invasion and capillary morphogenesis *in vitro*, and inhibits bFGF-induced angiogenesis (Anand-Apte *et al*. 1997). In ARMD eyes, TIMP-3 distribution in Bruch's membrane was abundant in areas of continuous soft drusen but absent in areas below RPE atrophy (Kamei and Hollyfield 1999).

Studies have also focussed on the role of integrins, specifically alpha versus beta 3 and alpha versus beta 5 and their role in ocular angiogenesis. Only alpha versus beta 3 was observed on blood vessels in ocular tissues with active neovascularization from patients with ARMD whereas both were presented in other disease states such as DR. This indicates that certain integrins are specific to certain disease states (Friedlander *et al*. 1996). A number of studies have shown that antagonists of integrins reduce CNV in ARMD (Honda *et al*. 2009). There is new evidence pointing to the involvement of ADAMs, proteins that have a disintegrin in addition to a metalloproteinase domain. They are in amplification loops with VEGF and further increase CNV. This is a new area of exploration for intervention. (Xie *et al*. 2008).

Inflammation

While it was described about two decades ago early in ARMD research, the role of inflammatory cells such as macrophages, dendritic cells and microglia and complement are once again being explored in the recent years (Penfold *et al*. 1987). The RPE produces a cytokine, MCP-1, monocyte chemotatic protein which recuits macrophages. Macrophages produce tissue factor, which in turn produce fibrin for infrastructure. These macrophages produce more VEGF along with the RPE (Grossniklaus *et al*. 2002). We see that inflammatory cells play a crucial role in ARMD as neutropenic mice show significantly less CNV in murine models. Neutrophils also contribute as they play an early role peaking at day three and produce other angiogenic factors in addition to VEGF (Zhou *et al*. 2005). Immature dendritic cells also play a role; they produce VEGFR-2 proliferate and migrate to areas of neovascularization from day 2–4 of CNV (Nakai *et al*. 2008).

In MCP-1 (*ccl-1*/*ccr-2*) knockout mice, macrophages and CNV were reduced, and the macrophages produced mRNA for VEGF, b-FBF, TNF-alpha and inflammatory molecules such as CD40, B7-1 and B7-2 (Tsutsumi *et al*. 2003), It seems that generalized depletion of macrophage activity decreases CNV (Sakurai *et al*. 2003a). However, other studies have shown contradictory evidence. In another MCP-1(*ccl-1*/*ccr-2*) deficient mouse model, there was a higher accumulation of complement immune complexes, drusen and lipofuscin. Macrophage recruitment by RPE may actually aid in decreasing the accumulation of material between the RPE and choroid (Ambati *et al*. 2003).

Microglia are also activated in response to rodphotoreceptor death and are involved in phagocytosing the injured and dead cells. In this process they may also cause nearby cone death. Any dysfunction in phagocytosis by microglia can lead to accumulation of cellular debris and activation of further inflammatory process leading to CNV (Gupta *et al*. 2003) An association between ARMD and CXC3R1, a chemokine produced by microglia cells (MC), has also been observed (Combadiere *et al*. 2007). Lower expression of, mutation of, or a reduction in CX3CR1-induced cellular activities may contribute to ARMD development due to the accumulation of MC and debris in the subretinal layer (Tuo *et al*. 2004; Combadiere *et al*. 2007). A study of an animal model deficient in both MCP-1 and CX3CR1 revealed severe progression to ARMD. Fifteen per cent of these mice had CNV and all showed increase in cellular debris, A2E, misfolded endoplasmic reticulum and chaperone proteins that accummulated subretinally (Ross *et al*. 2007).

Newer models of ARMD are moving towards the comparison of ARMD to cardiovascular disease. The E4 allele of apolipoprotein E has has been shown to be protective for ARMD, whereas the E2 allele has shown elevated risk. Apolipoprotein E is involved in the metabolism of cholesterol and lipid transport, thus ARMD has been compared to an atherosclerotic pathogenesis. ApoE112R decreases ARMD by suppressing VEGF and CCl-2 expression in the RPE (Bojanowski *et al*. 2006). Similar to atherosclerosis, a study with ARED's participants showed C-reactive protein was high in those with ARMD, showing that inflammation plays a role in both diseases (Seddon *et al*. 2004).

Complement

A constant low level of complement activation in the eye serves as a primary defence mechanism against pathogens and is tightly regulated by complement regulatory proteins (Bora *et al*. 2008). Recent studies suggest that dysregulation of the system may mediate ARMD pathogensis (Patel and Chan 2008). Components of all complement structures such as C5b–C9 and the MAC complex have been isolated in drusen of ARMD eyes (Mullins *et al*. 2000; Nozaki *et al*. 2006). It appears that it is the alternate pathway that is activated; in animal models inhibition of C4 and C1q had no effect in reducing CNV (Bora *et al*. 2006).

ARMD has been linked genetically and pathophysiologically with complement regulators and accessory molecules. For example, a knockout model of CD 59, a complement regulator, increased CNV and these effects were reduced when administered exogenous CD59 (Bora *et al*. 2007). There are several genetic variations of complement regulators that make one susceptible to ARMD. Chromosomal abnormalities that have been implicated thus far are 1q31-2, 6q21 and 10q26 (Gold *et al*. 2006; Swaroop *et al*. 2007).

CFH, a negative regulator of the complement system and c-reactive protein (CRP) (Prosser *et al*. 2007; Yates *et al*. 2007) can be found in several ocular tissues such as the cornea, retina, choroid and RPE. A tyrosine-to-histidine change in position Y402 in the 1q32 region of CFH gene is associated with increased ARMD. This change reduced affinity of binding capactiy to glycosaminoglycans (GAG) and CRP, and dysregulation of the alternative pathyway. CFH deficient mice have C3 deposition and disorganized photoreceptors and

even display signs of higher oxidative damage (Klein *et al*. 2005). The possession of at least one histidine at amino acid 402 increases the risk of ARMD by 2.7. Some have insisted that it accounts for 50% correlation with ARMD (Thakkinstian *et al*. 2006; Kleinman *et al*. 2008).

Within chromosome 6q21 are complement factor B and complement component 2 that are located 500 bp apart. These are complement activators located in MHC III region. Variants of BF, R32QBF/a and L9HBF and CC2; E318D in intron 10 of C2 are all associated with reduced ARMD, predicting clinical outcome in 74% of those affected. The protective effect is likely due to decreased enzymatic activity in complement response. Though the direct mechanism is still unknown, animals models with knockout BF have shown reduced levels of CNV (Gold *et al*. 2006). However, later studies have not shown similar results. While they show an association with ARMD, it is not as high as that of CFH or HtrA1 and the researchers have attributed the association to other unidentified SNPs located in that region and due to their strong linkage disequilibrium (Spencer *et al*. 2007; McKay *et al*. 2009).

Within chromosome 10q26 is *HtrA1*, a gene for a heat shock serine protease that is upregulated during cellular stress and inhibits the Tgf-beta family of proteins by blocking receptor activation (Oka *et al*. 2004). *HtrD1* is constitutively expressed in ocular tissues for normal eye development but is highly expressed in those with ARMD, wet>dry (Oka *et al*. 2004; Chan *et al*. 2007). It is thought that HtrA1 induces apoptosis and degradation of the ECM proteins. An SNP, rs11200638, in the promoter of *HtrA1* has a high association with wet ARMD and shows increased levels of *HtrA1* mRNA and protein in affected individuals (Chan *et al*. 2007). Recently, another polymorphism 512G>A, has also been shown to be associated with ARMD (Tang *et al*. 2009). Two other alleles, PLEKHA1 and LOC387715 next to HtrA1 are strongly associated with ARMD susceptibility (Swaroop *et al*. 2007; Ross *et al*. 2007). SNP rs1045216 in PLEKHA is associated with increased CNV as is rs10490924 in the hypothetical LOC387715/ARMS2 gene (Conley *et al*. 2006). LOC387715/ARMS2 and PLEKHA1 maybe involved in intracellular remodelling and lymphocytic activation (Swaroop *et al*. 2007; Ross *et al*. 2007).

Variations in C3 at chromosome 19p13 have been associated with ARMD. C3 is a main component of the complement cascade and its cleavage products have been found in drusen (Nozaki *et al*. 2006). Its deficiency reduces angiogenic factors such as VEGF, TGF-B2 and B-FGF in the eye (Nozaki *et al*. 2006; Bora *et al*. 2006). Studies have shown that a certain variation in SNPs in this complement factor and have been associated with ARMD, particularly a variation of one amino acid at 80(R80G) (Yates *et al*. 2007). A more recent study showed two other variants, rs22030199(R102G) and rs1047286 (P314L) also associated with ARMD; the changes in sequences altered binding to pathogenic cells and other complement factors (Despriet *et al*. 2009).

Toll like receptors (TLR) are involved in mounting an immune response to a foreign pathogen. Thus far ones that have been implicated are Tlr7 which recognized single stranded DNA, Tlr4, recognizing lipopolysaccharide, and Tlr3 which recognizes double stranded RNA, the last has been found to have the most association with geographic atrophy in ARMD (Edwards *et al*. 2008; Yang *et al*. 2008). It is theorized that intracellular transmission of viral transcripts may activate Tlr3 and trigger inflammatory cascades leading to apoptosis and cell death of the RPE (Edwards *et al*. 2008). The phe variation of this receptor suppresses dsRNA mediated atrophy by inducing less apoptosis than the Leu–Leu variant (Yang *et al*. 2008). While there is no direct association between variation in Tlr3 to CNV, recent studies have shown that siRNA therapy suppresses CNV via Tlr3, showing that there may be a role of Tlr3 activation in reducing CNV (Kleinman *et al*. 2008). Table 3 summarizes factors involved in modulating retinal angiogenesis.

Conclusion

Over the past decade, novel markers of neovascularization have been identified, both at a molecular and genetic levels, consequently leading our understanding of the molecular mechanisms involved in ocular neovascularization to new heights. Stemming from better insight into the complex interplay of molecules in ocular angiogenesis, we believe that a multi-faceted approach to retarding or curbing NV is needed; targeting an array of biomolecules and modulating multiple signalling cascades holds promise for efficacious control of NV. Of late, gene therapy has received increasing attention from scientists around the world. We believe that the answer may lie in manipulating transcription factors and alternative splicing of putative genes involved in ocular NV, tipping the microenvironment to an anti-angiogenic state. We believe that through techniques in gene therapy, alternative splicing and RNA interference, we may meet with greater success in restoring ocular 'angiogenic privilege'.

Acknowledgments

This publication was made possible by funds from RPB Physician Scientist Award (BKA), NEI grants 5R01EY017950 and NEI 5R01EY01782 (BKA).

References

Abdiu O, Van Setten G. Antiangiogenic activity in tears: presence of pigment-epithelium-derived factor. New insights and preliminary results? Ophthalmic Res. 2008; 40:16–18. [PubMed: 18032913]

Adamis AP, Berman AJ. Immunological mechanisms in the pathogenesis of diabetic retinopathy. Semin. Immunopathol. 2008; 30:65–84. [PubMed: 18340447]

Adamis AP, Miller JW, Bernal MT, D'Amico DJ, Folkman J, Yeo TK, et al. Increased vascular endothelial growth factor levels in the vitreous of eyes with diabetic retinopathy. Am. J. Ophthalmol. 1994; 118:445–450. [PubMed: 7943121]

Alon T, Hemo I, Itin A, Pe'er J, Stone J, Keshet E. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. Nat. Med. 1995; 1:1024–1028. [PubMed: 7489357]

Ambati BK, Joussen AM, Ambati J, Moromizato Y, Guha C, Javaherian K, et al. Angiostatin inhibits and regresses corneal neovascularization. Arch. Ophthalmol. 2002; 120:1063–1068. [PubMed: 12149060]

Ambati BK, Anand A, Joussen AM, Kuziel WA, Adamis AP, Ambati J. Sustained inhibition of corneal neovascularization by genetic ablation of CCR5. Invest. Ophthalmol. Vis. Sci. 2003a; 44:590–593. [PubMed: 12556387]

Ambati BK, Joussen AM, Kuziel WA, Adamis AP, Ambati J. Inhibition of corneal neovascularization by genetic ablation of CCR2. Cornea. 2003b; 22:465–467. [PubMed: 12827053]

Ambati J, Anand A, Fernandez S, Sakurai E, Lynn BC, Kuziel WA, et al. An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2- deficient mice. Nat. Med. 2003c; 9:1390–1397. [PubMed: 14566334]

Ambati BK, Nozaki M, Singh N, Takeda A, Jani PD, Suthar T, et al. Corneal avascularity is due to soluble VEGF receptor1. Nature. 2006; 443:993–997. [PubMed: 17051153]

Amin R, Puklin JE, Frank RN. Growth factor localization in choroidal neovascular membranes of age-related macular degeneration. Invest. Ophthalmol. Vis. Sci. 1994; 35:3178–3188. [PubMed: 7519180]

Anand-Apte B, Pepper MS, Voest E, Montesano R, Olsen B, Murphy G, et al. Inhibition of angiogenesis by tissue inhibitor of metalloproteinase-3. Invest. Ophthalmol. Vis. Sci. 1997; 38:817–823. [PubMed: 9112976]

Ando A, Yang A, Nambu H, Campochiaro PA. Blockade of nitric-oxide synthase reduces choroidal neovascularization. Mol. Pharmacol. 2002; 62:539–544. [PubMed: 12181430]

Apte SS, Mattei MG, Olsen BR. Cloning of the cDNA encoding human tissue inhibitor of metalloproteinases-3 (TIMP-3) and mapping of the TIMP3 gene to chromosome 22. Genomics. 1994; 19:86–90. [PubMed: 8188246]

Apte RS, Barreiro RA, Duh E, Volpert O, Ferguson TA. Stimulation of neovascularization by the anti-angiogenic factor PEDF. Invest. Ophthalmol. Vis. Sci. 2004; 45:4491–4497. [PubMed: 15557459]

Arden GB, Sidman RL, Arap W, Schlingemann RO. Spare the rod and spoil the eye. Br. J. Ophthalmol. 2005; 89:764–769. [PubMed: 15923516]

Arjamaa O, Nikinmaa M. Oxygen-dependent diseases in the retina: role of hypoxia-inducible factors. Exp. Eye Res. 2006; 83:473–483. [PubMed: 16750526]

Ashton N, Ward B, Serpell G. Effect of oxygen on developing retinal vessels with particular reference to the problem of retrolental fibroplasia. Br. J. Ophthalmol. 1954; 38:397–432. [PubMed: 13172417]

Azar DT. Corneal angiogenic privilege: angiogenic and antiangiogenic factors in corneal avascularity, vasculogenesis, and wound healing (an American Ophthalmological Society thesis). Trans. Am. Ophthalmol. Soc. 2006; 104:264–302. [PubMed: 17471348]

Azar DT, Hahn TW, Jain S, Yeh YC, Stetler-Stevensen WG. Matrix metalloproteinases are expressed during wound healing after excimer laser keratectomy. Cornea. 1996; 15:18–24. [PubMed: 8907376]

Becerra SP, Amaral J. Erythropoietin - an endogenous retinal survival factor. N. Engl. J. Med. 2002; 347:1968–1970. [PubMed: 12477950]

Beck L Jr, D'Amore PA. Vascular development: cellular and molecular regulation. FASEB J. 1997; 11:365–373. [PubMed: 9141503]

Beranek M, Kolar P, Tschoplova S, Kankova K, Vasku A. Genetic variations and plasma levels of gelatinase A (matrix metalloproteinase-2) and gelatinase B (matrix metalloproteinase-9) in proliferative diabetic retinopathy. Mol. Vis. 2008; 14:1114–1121. [PubMed: 18552985]

Berglin L, Sarman S, van der Ploeg I, Steen B, Ming Y, Itohara S, et al. Reduced choroidal neovascular membrane formation in matrix metalloproteinase-2- deficient mice. Invest. Ophthalmol. Vis. Sci. 2003; 44:403–408. [PubMed: 12506102]

Bhutto IA, McLeod DS, Hasegawa T, Kim SY, Merges C, Tong P, et al. Pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) in aged human choroid and eyes with age-related macular degeneration. Exp. Eye Res. 2006; 82:99–110. [PubMed: 16019000]

Bikfalvi A, Han ZC. Angiogenic factors are hematopoietic growth factors and vice versa. Leukemia. 1994; 8:523–529. [PubMed: 7510358]

Blaauwgeers HG, Holtkamp GM, Rutten H, Witmer AN, Koolwijk P, Partanen TK, et al. Polarized vascular endothelial growth factor secretion by human retinal pigment epithelium and localization of vascular endothelial growth factor receptors on the inner choriocapillaris. Evidence for a trophic paracrine relation. Am. J. Pathol. 1999; 155:421–428. [PubMed: 10433935]

Boehm B, Lang G, Feldmann B, Kurkhaus A, Rosinger S, Volpert O, et al. Proliferative diabetic retinopathy is associated with a low level of the natural ocular anti-angiogenic agent pigment epithelium-derived factor (PEDF) in aqueous humor. a pilot study. Horm. Metab. Res. 2003; 35:382–386. [PubMed: 12920663]

Bojanowski CM, Shen D, Chew EY, Ning B, Csaky KG, Green WR, et al. An apolipoprotein E variant may protect against age-related macular degeneration through cytokine regulation. Environ. Mol. Mutagen. 2006; 47:594–602. [PubMed: 16823865]

Bora NS, Kaliappan S, Jha P, Xu Q, Sohn JH, Dhaulakhandi DB, et al. Complement activation via alternative pathway is critical in the development of laser-induced choroidal neovascularization: role of factor B and factor H. J. Immunol. 2006; 177:1872–1878. [PubMed: 16849499]

Bora NS, Kaliappan S, Jha P, Xu Q, Sivasankar B, Harris CL, et al. CD59, a complement regulatory protein, controls choroidal neovascularization in a mouse model of wet-type age-related macular degeneration. J. Immunol. 2007; 178:1783–1790. [PubMed: 17237428]

Bora NS, Jha P, Bora PS. The role of complement in ocular pathology. Semin. Immunopathol. 2008; 30:85–95. [PubMed: 18299835]

Brown MS, Baron AE, France EK, Hamman RF. Association between higher cumulative doses of recombinant erythropoietin and risk for retinopathy of prematurity. J. AAPOS. 2006; 10:143–149. [PubMed: 16678749]

Browning AC, Dua HS, Amoaku WM. The effects of growth factors on the proliferation and in vitro angiogenesis of human macular inner choroidal endothelial cells. Br. J. Ophthalmol. 2008; 92:1003–1008. [PubMed: 18577655]

Buraczynska M, Ksiazek P, Baranowicz-Gaszczyk I, Jozwiak L. Association of the VEGF gene polymorphism with diabetic retinopathy in type 2 diabetes patients. Nephrol. Dial. Transplant. 2007; 22:827–832. [PubMed: 17121786]

Burger PC, Chandler DB, Klintworth GK. Corneal neovascularization as studied by scanning electron microscopy of vascular casts. Lab. Invest. 1983; 48:169–180. [PubMed: 6185761]

Burger PC, Chandler DB, Klintworth GK. Experimental corneal neovascularization: biomicroscopic, angiographic, and morphologic correlation. Cornea. 1985; 4:35–41. [PubMed: 2419029]

Campochiaro PA. Ocular neovascularisation and excessive vascular permeability. Expert Opin. Biol. Ther. 2004; 4:1395–1402. [PubMed: 15335307]

Campochiaro PA. Potential applications for RNAi to probe pathogenesis and develop new treatments for ocular disorders. Gene Ther. 2006; 13:559–562. [PubMed: 16195702]

Cao R, Wu HL, Veitonmaki N, Linden P, Farnebo J, Shi GY, et al. Suppression of angiogenesis and tumor growth by the inhibitor K1-5 generated by plasmin-mediated proteolysis. Proc. Natl. Acad. Sci. USA. 1999; 96:5728–5733. [PubMed: 10318952]

Carmeliet P. Angiogenesis in health and disease. Nat. Med. 2003; 9:653–660. [PubMed: 12778163]

Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gert-senstein M, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature. 1996; 380:435–439. [PubMed: 8602241]

Chan CC, Shen D, Zhou M, Ross RJ, Ding X, Zhang K, et al. Human HtrA1 in the archived eyes with age-related macular degeneration. Trans. Am. Ophthalmol. Soc. 2007; 105:92–97. discussion 97–98. [PubMed: 18427598]

Chang JH, Gabison EE, Kato T, Azar DT. Corneal neovascularization. Curr. Opin. Ophthalmol. 2001; 12:242–249. [PubMed: 11507336]

Chan-Ling T, Gock B, Stone J. The effect of oxygen on vasoformative cell division. Evidence that 'physiological hypoxia' is the stimulus for normal retinal vasculogenesis. Invest. Ophthalmol. Vis. Sci. 1995; 36:1201–1214. [PubMed: 7775098]

Chen J, Smith LE. Retinopathy of prematurity. Angiogenesis. 2007; 10:133–140. [PubMed: 17332988]

Chen J, Hicks D, Brantley-Sieders D, Cheng N, McCollum GW, Qiwerdich X, et al. Inhibition of retinal neovascularization by soluble EphA2 receptor. Exp. Eye. Res. 2006; 82:664–673. [PubMed: 16359662]

Chen J, Connor KM, Aderman CM, Smith LE. Erythropoietin deficiency decreases vascular stability in mice. J. Clin. Invest. 2008; 118:526–533. [PubMed: 18219389]

Chen J, Connor KM, Aderman CM, Willett KL, Aspegren OP, Smith LE. Suppression of retinal neovascularization by erythropoietin siRNA in a mouse model of proliferative retinopathy. Invest. Ophthalmol. Vis. Sci. 2009; 50:1329–1335. [PubMed: 18952918]

Cheung AK, Fung MK, Lo AC, Lam TT, So KF, Chung SS, et al. Aldose reductase deficiency prevents diabetesinduced blood-retinal barrier breakdown, apoptosis, and glial reactivation in the retina of db/db mice. Diabetes. 2005; 54:3119–3125. [PubMed: 16249434]

Chmielewska K, Robaszkiewicz J, Kosatka M. Role of the retinal pigment epithelium (RPE) in the pathogenesis and treatment of diabetic macular edema (DME). Klin. Oczna. 2008; 110:318–320. [PubMed: 19112870]

Churchill AJ, Carter JG, Ramsden C, Turner SJ, Yeung A, Brenchley PE, et al. VEGF polymorphisms are associated with severity of diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 2008; 49:3611–3616. [PubMed: 18441306]

Cohen MP, Hud E, Shea E, Shearman CW. Vitreous fluid of db/db mice exhibits alterations in angiogenic and metabolic factors consistent with early diabetic retinopathy. Ophthalmic Res. 2008; 40:5–9. [PubMed: 18025835]

Combadiere C, Feumi C, Raoul W, Keller N, Rodero M, Pezard A, et al. CX3CR1-dependent subretinal microglia cell accumulation is associated with cardinal features of age-related macular degeneration. J. Clin. Invest. 2007; 117:2920–2928. [PubMed: 17909628]

Conley YP, Jakobsdottir J, Mah T, Weeks DE, Klein R, Kuller L, et al. CFH, ELOVL4, PLEKHA1 and LOC387715 genes and susceptibility to age- related maculopathy:AREDS and CHS cohorts and meta-analyses. Hum. Mol. Genet. 2006; 15:3206–3218. [PubMed: 17000705]

Connor KM, SanGiovanni JP, Lofqvist C, Aderman CM, Chen J, Higuchi A, et al. Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. Nat. Med. 2007; 13:868–873. [PubMed: 17589522]

Crawford TN, Alfaro DV 3rd, Kerrison JB, Jablon EP. Diabetic retinopathy and angiogenesis. Curr. Diabetes Rev. 2009; 5:8–13. [PubMed: 19199892]

Cross MJ, Claesson-Welsh L. FGF and VEGF function in angiogenesis: signalling pathways, biological responses and therapeutic inhibition. Trends Pharmacol. Sci. 2001; 22:201–207. [PubMed: 11282421]

Cursiefen C, Kuchle M, Naumann GO. Angiogenesis in corneal diseases: histopathologic evaluation of 254 human corneal buttons with neovascularization. Cornea. 1998; 17:611–613. [PubMed: 9820941]

Dawson DW, Volpert OV, Gillis P, Crawford SE, Xu H, Benedict W, et al. Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. Science. 1999; 285:245–248. [PubMed: 10398599]

Demircan N, Safran BG, Soylu M, Ozcan AA, Sizmaz S. Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy. Eye. 2006; 20:1366–1369. [PubMed: 16284605]

Despriet DD, van Duijn CM, Oostra BA, Uitterlinden AG, Hofman A, Wright F, et al. Complement component C3 and risk of age-related macular degeneration. Ophthalmology. 2009; 116:474–480. e472. [PubMed: 19168221]

Economopoulou M, Bdeir K, Cines DB, Fogt F, Bdeir Y, Lubkowski J, et al. Inhibition of pathologic retinal neovascularization by alpha-defensins. Blood. 2005; 106:3831–3838. [PubMed: 16123222]

Edwards AO, Chen D, Fridley BL, James KM, Wu Y, Abecasis G, et al. Toll-like receptor polymorphisms and age-related macular degeneration. Invest. Ophthalmol. Vis. Sci. 2008; 49:1652–1659. [PubMed: 18385087]

Fang IM, Yang CH, Yang CM, Chen MS. Comparative effects of fatty acids on proinflammatory gene cyclooxygenase 2 and inducible nitric oxide synthase expression in retinal pigment epithelial cells. Mol. Nutr. Food Res. 2009; 53:739–750. [PubMed: 19437483]

Ferreras M, Felbor U, Lenhard T, Olsen BR, Delaisse J. Generation and degradation of human endostatin proteins by various proteinases. FEBS Lett. 2000; 486:247–251. [PubMed: 11119712]

Folkman J. Tumor angiogenesis: therapeutic implications. N. Engl. J. Med. 1971; 285:1182–1186. [PubMed: 4938153]

Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat. Med. 1995; 1:27–31. [PubMed: 7584949]

Friedlander M, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, Cheresh A. Definition of two angiogenic pathways by distinct alpha v integrins. Science. 1995; 270:1500–1502. [PubMed: 7491498]

Friedlander M, Theesfeld CL, Sugita M, Fruttiger M, Thomas MA, Chang S, Cheresh DA. Involvement of integrins alpha v beta 3 and alpha v beta 5 in ocular neovascular diseases. Proc. Natl. Acad. Sci. USA. 1996; 93:9764–9769. [PubMed: 8790405]

Fukumura D, Gohongi T, Kadambi A, Izumi Y, Ang J, Yun CO, et al. Predominant role of endothelial nitric oxide synthase in vascular endothelial growth factor-induced angiogenesis and vascular permeability. Proc. Natl. Acad. Sci. USA. 2001; 98:2604–2609. [PubMed: 11226286]

Funatsu H, Yamashita H, Noma H, Mimura T, Nakamura S, Sakata K, et al. Aqueous humor levels of cytokines are related to vitreous levels and progression of diabetic retinopathy in diabetic patients. Graefes. Arch. Clin. Exp. Ophthalmol. 2005; 243:3–8. [PubMed: 15258777]

Gao G, Li Y, Zhang D, Gee S, Crosson C, Ma J. Unbalanced expression of VEGF and PEDF in ischemia-induced retinal neovascularization. FEBS Lett. 2001; 489:270–276. [PubMed: 11165263]

Gerritsen ME, Soriano R, Yang S, Zlot C, Ingle G, Toy K, et al. Branching out: a molecular fingerprint of endothelial differentiation into tube-like structures generated by Affymetrix oligonucleotide arrays. Microcirculation. 2003; 10:63–81. [PubMed: 12610664]

Gold B, Merriam JE, Zernant J, Hancox LS, Taiber AJ, Gehrs K, et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. Nat. Genet. 2006; 38:458–462. [PubMed: 16518403]

Grant MB, Afzal A, Spoerri P, Pan H, Shaw LC, Mames RN. The role of growth factors in the pathogenesis of diabetic retinopathy. Expert Opin. Investig. Drugs. 2004; 13:1275–1293.

Grossniklaus HE, Ling JX, Wallace TM, Dithmar S, Lawson DH, Cohen C, et al. Macrophage and retinal pigment epithelium expression of angiogenic cytokines in choroidal neovascularization. Mol. Vis. 2002; 8:119–126. [PubMed: 11979237]

Guo L, Hussain AA, Limb GA, Marshall J. Age-dependent variation in metalloproteinase activity of isolated human Bruch's membrane and choroid. Invest. Ophthalmol. Vis. Sci. 1999; 40:2676–2682. [PubMed: 10509665]

Gupta N, Brown KE, Milam AH. Activated microglia in human retinitis pigmentosa, late-onset retinal degeneration, and age-related macular degeneration. Exp. Eye Res. 2003; 76:463–471. [PubMed: 12634111]

Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell. 1996; 86:353–364. [PubMed: 8756718]

Hangai M, Moon YS, Kitaya N, Chan CK, Wu DY, Peters KG, et al. Systemically expressed soluble Tie2 inhibits intraocular neovascularization. Hum. Gene. Ther. 2001; 12:1311–1321. [PubMed: 11440624]

Hattenbach LO, Falk B, Nurnberger F, Koch FH, Ohrloff C. Detection of inducible nitric oxide synthase and vascular endothelial growth factor in choroidal neovascular membranes. Ophthalmologica. 2002; 216:209–214. [PubMed: 12065859]

Hellstrom A, Perruzzi C, Ju M, Engstrom E, Hard AL, Liu JL, et al. Low IGF-I suppresses VEGF-survival signaling in retinal endothelial cells: direct correlation with clinical retinopathy of prematurity. Proc. Natl. Acad. Sci. USA. 2001; 98:5804–5808. [PubMed: 11331770]

Hellstrom A, Engstrom E, Hard AL, Albertsson-Wikland K, Carlsson B, Niklasson A, et al. Postnatal serum insulinlike growth factor I deficiency is associated with retinopathy of prematurity and other complications of premature birth. Pediatrics. 2003; 112:1016–1020. [PubMed: 14595040]

He T, Ai M, Zhao XH, Xing YQ. Inducible nitric oxide synthase mediates hypoxia-induced hypoxia-inducible factor-1 alpha activation and vascular endothelial growth factor expression in oxygen-induced retinopathy. Pathobiology. 2007; 74:336–343. [PubMed: 18087198]

Hiratsuka S, Kataoka Y, Nakao K, Nakamura K, Morikawa S, Tanaka S, et al. Vascular endothelial growth factor A (VEGF-A) is involved in guidance of VEGF receptor-positive cells to the anterior portion of early embryos. Mol. Cell Biol. 2005; 25:355–363. [PubMed: 15601856]

Hoffmann S, He S, Ehren M, Ryan SJ, Wiedemann P, Tanaka S, et al. MMP-2 and MMP-9 secretion by rpe is stimulated by angiogenic molecules found in choroidal neovascular membranes. Retina. 2006; 26:454–461. [PubMed: 16603966]

Holekamp NM, Bouck N, Volpert O. Pigment epithelium-derived factor is deficient in the vitreous of patients with choroidal neovascularization due to age-related macular degeneration. Am. J. Ophthalmol. 2002; 134:220–227. [PubMed: 12140029]

Honda S, Nagai T, Negi A. Anti-angiogenic effects of non-peptide integrin alphavbeta3 specific antagonist on laser-induced choroidal neovascularization in mice. Graefes Arch. Clin. Exp. Ophthalmol. 2009; 247:515–522. [PubMed: 19048271]

Hughes S, Yang H, Chan-Ling T. Vascularization of the human fetal retina: roles of vasculogenesis and angiogenesis. Invest. Ophthalmol. Vis. Sci. 2000; 41:1217–1228. [PubMed: 10752963]

Hughes JM, Kuiper EJ, Klaassen I, Canning P, Stitt AW, Van Bezu J, et al. Advanced glycation and products casue increased CCN family and extracellular matrix gene expression in the diabetic rodent retina. Diabetologia. 2007; 50:1089–1098. [PubMed: 17333105]

Hutchings H, Maitre-Boube M, Tombran-Tink J, Plouet J. Pigment epithelium-derived factor exerts opposite effects on endothelial cells of different phenotypes. Biochem. Biophys. Res. Commun. 2002; 294:764–769. [PubMed: 12061772]

Iizuka H, Awata T, Osaki M, Neda T, Kurihara S, Inoue K, et al. Promoter polymorphisms of the pigment epitheliumderived factor gene are associated with diabetic retinopathy. Biochem. Biophys. Res. Commun. 2007; 361:421–426. [PubMed: 17658465]

Iliaki E, Poulaki V, Mitsiades N, Mitsiades CS, Miller JW, Gragoudas ES. Role of {alpha}4 integrin (cd49d) in the pathogenesis of diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 2009; 50:4890–4904.

Ishida S. Lifestyle-related diseases and anti-aging ophthalmology: suppression of retinal and choroidal pathologies by inhibiting renin-angiotensin system and inflammation. Nippon Ganka Gakkai Zasshi. 2009; 113:403–423. [PubMed: 19348185]

Isner JM, Asahara T. Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularization. J. Clin. Invest. 1999; 103:1231–1236. [PubMed: 10225965]

Itoh N, Ornitz DM. Evolution of the Fgf and Fgfr gene families. Trends Genet. 2004; 20:563–569. [PubMed: 15475116]

Itoh T, Tanioka M, Yoshida H, Yoshioka T, Nishimoto H, Itohara S. Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. Cancer Res. 1998; 58:1048–1051. [PubMed: 9500469]

Jani PD, Singh N, Jenkins C, Raghava S, Mo Y, Amin S, et al. Nanoparticles sustain expression of Flt intraceptors in the cornea and inhibit injury-induced corneal angiogenesis. Invest. Ophthalmol. Vis. Sci. 2007; 48:2030–2036. [PubMed: 17460257]

Jaquet K, Krause K, Tawakol-Khodai M, Geidel S, Kuck KH. Erythropoietin and VEGF exhibit equal angiogenic potential. Microvasc. Res. 2002; 64:326–333. [PubMed: 12204656]

Jiang J, Xia XB, Xu HZ, Xiong Y, Song WT, Xiong SQ, et al. Inhibition of retinal neovascularization by gene transfer of small interfering RNA targeting HIF-1alpha and VEGF. J. Cell. Physiol. 2009; 218:66–74. [PubMed: 18767037]

Jones CA, London NR, Chen H, Park KW, Sauvaget D, Stockton RA, et al. Robo4 stabilizes the vascular network by inhibiting pathologic angiogenesis and endothelial hyperpermeability. Nat. Med. 2008; 14:448–453. [PubMed: 18345009]

Kadonosono K, Yazama F, Itoh N, Sawada H, Ohno S. Expression of matrix metalloproteinase-7 in choroidal neovascular membranes in age-related macular degeneration. Am. J. Ophthalmol. 1999; 128:382–384. [PubMed: 10511046]

Kamei M, Hollyfield JG. TIMP-3 in Bruch's membrane: changes during aging and in age-related macular degeneration. Invest. Ophthalmol. Vis. Sci. 1999; 40:2367–2375. [PubMed: 10476804]

Kaneko T, Fujii S, Matsumoto A, Goto D, Ishimori N, Watano K, et al. Induction of plasminogen activator inhibitor-1 in endothelial cells by basic fibroblast growth factor and its modulation by fibric acid. Arterioscler. Thromb. Vasc. Biol. 2002; 22:855–860. [PubMed: 12006402]

Karakousis PC, John SK, Behling KC, Surace EM, Smith JE, Hendrickson A, et al. Localization of pigment epithelium derived factor (PEDF) in developing and adult human ocular tissues. Mol. Vis. 2001; 7:154–163. [PubMed: 11438800]

Keck PJ, Hauser SD, Krivi G, Sanzo K, Warren T, Feder J, et al. Vascular permeability factor, an endothelial cell mitogen related to PDGF. Science. 1989; 246:1309–1312. [PubMed: 2479987]

Kern TS. Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. Exp. Diabetes Res. 2007:95103. [PubMed: 18274606]

Kim I, Moon SO, Park SK, Chae SW, Koh GY. Angiopoietin-1 reduces VEGF-stimulated leukocyte adhesion to endothelial cells by reducing ICAM-1, VCAM-1, and E-selectin expression. Circ. Res. 2001; 89:477–479. [PubMed: 11557733]

Kim JH, Yu YS, Cho CS, Kim KW. Blockade of angiotensin II attenuates VEGF-mediated blood-retinal barrier breakdown in diabetic retinopathy. J. Cereb. Blood Flow Metab. 2009; 29:621–628. [PubMed: 19107135]

Kita T, Hata Y, Miura M, Kawahara S, Nakao S, Ishibashi T. Functional characteristics of connective tissue growth factor on vitreoretinal cells. Diabetes. 2007; 56:1421–1428. [PubMed: 17303801]

Kitaoka T, Morse LS, Schneeberger S, Ishigooka H, Hjelme-land LM. Expression of FGF5 in choroidal neovascular membranes associated with ARMD. Curr. Eye Res. 1997; 16:396–399. [PubMed: 9134330]

Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, et al. Complement factor H polymorphism in age-related macular degeneration. Science. 2005; 308:385–389. [PubMed: 15761122]

Klein S, Giancotti FG, Presta M, Albelda SM, Buck CA, Rifkin DB. Basic fibroblast growth factor modulates integrin expression in microvascular endothelial cells. Mol. Biol. Cell. 1993; 4:973–982. [PubMed: 8298194]

Kleinman ME, Yamada K, Takeda A, Chandrasekaran V, Nozaki M, Baffi JZ, et al. Sequence- and target-independent angiogenesis suppression by siRNA via TLR3. Nature. 2008; 452:591–597. [PubMed: 18368052]

Krantz SB. Erythropoietin. Blood. 1991; 77:419–434. [PubMed: 1991159]

Kuiper EJ, Van Nieuwenhoven FA, de Smet MD, van Meurs JC, Tanck MW, Oliver N, et al. The angiofibrotic switch of VEGF and CTGF in proliferative diabetic retinopathy. PLoS One. 2008; 3:e2675. [PubMed: 18628999]

Kvanta A. Ocular angiogenesis: the role of growth factors. Acta Ophthalmol. Scand. 2006; 84:282–288. [PubMed: 16704684]

Kvanta A, Shen WY, Sarman S, Seregard S, Steen B, Rakoczy E. Matrix metalloproteinase (MMP) expression in experimental choroidal neovascularization. Curr. Eye Res. 2000; 21:684–690. [PubMed: 11120556]

Langford K, Nicolaides K, Miell JP. Maternal and fetal insulin-like growth factors and their binding proteins in the second and third trimesters of human pregnancy. Hum. Reprod. 1998; 13:1389–1393. [PubMed: 9647578]

Lee P, Wang CC, Adamis AP. Ocular neovascularization: an epidemiologic review. Surv. Ophthalmol. 1998; 43:245–269. [PubMed: 9862312]

Lee PC, Kibbe MR, Schuchert MJ, Stolz DB, Watkins SC, Griffith BP, et al. Nitric oxide induces angiogenesis and upregulates alpha(v)beta(3) integrin expression on endothelial cells. Microvasc. Res. 2000; 60:269–280. [PubMed: 11078643]

Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science. 1989; 246:1306–1309. [PubMed: 2479986]

Lin HC, Chang JH, Jain S, Gabison EE, Kure T, Kato T, et al. Matrilysin cleavage of corneal collagen type XVIII NC1 domain and generation of a 28-kDa fragment. Invest. Ophthalmol. Vis. Sci. 2001; 42:2517–2524. [PubMed: 11581192]

Lin JM, Wan L, Tsai YY, Lin HJ, Tsai Y, Lee CC, et al. Pigment epithelium-derived factor gene Met72Thr polymorphism is associated with increased risk of wet age-related macular degeneration. Am. J. Ophthalmol. 2008; 145:716–721. [PubMed: 18226801]

Lip PL, Chatterjee S, Caine GJ, Hope-Ross M, Gibson J, Blann AD, et al. Plasma vascular endothelial growth factor, angiopoietin-2, and soluble angiopoietin receptor Tie-2 in diabetic retinopathy: effects of laser photocoagulation and angiotensin receptor blockade. Br. J. Ophthalmol. 2004; 88:1543–1546. [PubMed: 15548809]

Liu J, Lin TH, Cole AG, Wen R, Zhao L, Brescia MR, et al. Identification and characterization of small-molecule inhibitors of Tie2 kinase. FEBS Lett. 2008; 582:785–791. [PubMed: 18267118]

Lommatzsch A, Hermans P, Muller KD, Bornfeld N, Bird AC, Pauleikhoff D. Are low inflammatory reactions involved in exudative age-related macular degeneration? Morphological and immunhistochemical analysis of AMD associated with basal deposits. Graefes Arch. Clin. Exp. Ophthalmol. 2008; 246:803–810. [PubMed: 18414889]

Lu PC, Ye H, Maeda M, Azar DT. Immunolocalization and gene expression of matrilysin during corneal wound healing. Invest. Ophthalmol. Vis. Sci. 1999; 40:20–27. [PubMed: 9888422]

Lukiw WJ, Bazan NG. Docosahexaenoic acid and the aging brain. J. Nutr. 2008; 138:2510–2514. [PubMed: 19022980]

Madan A, Penn JS. Animal models of oxygen-induced retinopathy. Front. Biosci. 2003; 8:1030–1043.

Maeda M, Vanlandingham BD, Ye H, Lu PC, Azar DT. Immunoconfocal localization of gelatinase B expressed by migrating intrastromal epithelial cells after deep annular excimer keratectomy. Curr. Eye Res. 1998; 17:836–843. [PubMed: 9724000]

Mahabeleshwar GH, Feng W, Phillips DR, Byzova TV. Integrin signalling is critical for pathological angiogenesis. J. Exp. Med. 2006; 203:2495–2507. [PubMed: 17030947]

Malik RA, Li C, Aziz W, Olson JA, Vohra A, McHardy KC, et al. Elevated plasma CD105 and vitreous VEGF levels in diabetic retinopathy. J. Cell Mol. Med. 2005; 9:692–697. [PubMed: 16202216]

Matsunaga N, Chikaraishi Y, Izuta H, Ogata N, Shimazawa M, Matsumura M, et al. Role of soluble vascular endothelial growth factor receptor-1 in the vitreous in proliferative diabetic retinopathy. Ophthalmology. 2008; 115:1916–1922. [PubMed: 18718666]

Matsushima M, Ogata N, Takada Y, Tobe T, Yamada H, Taka-hashi K, et al. FGF receptor 1 expression in experimental choroidal neovascularization. Jpn. J. Ophthalmol. 1996; 40:329–338. [PubMed: 8988422]

Mattila MM, Ruohola JK, Valve EM, Tasanen MJ, Seppanen JA, Harkonen L. FGF-8b increases angiogenic capacity and tumor growth of androgen-regulated S115 breast cancer cells. Oncogene. 2001; 20:2791–2804. [PubMed: 11420691]

McKay GJ, Silvestri G, Patterson CC, Hogg RE, Chakravarthy U, Hughes AE. Further assessment of the complement component 2 and factor B region associated with agerelated macular degeneration. Invest. Ophthalmol. Vis. Sci. 2009; 50:533–539. [PubMed: 18806297]

McLeod DS, Taomoto M, Cao J, Zhu Z, Witte L, Lutty GA. Localization of VEGF receptor2 (KDR/Flk1) and effects of blocking it in oxygeninduced retinopathy. Invest. Ophthalmol. Vis . Sci. 2002; 43:474–482. [PubMed: 11818393]

Meleth AD, Agron E, Chan CC, Reed GF, Arora K, Byrnes G, et al. Serum inflammatory markers in diabetic retinopathy. Invest. Ophthalmol. Vis. Sci. 2005; 46:4295–4301. [PubMed: 16249511]

Mignatti P, Rifkin DB. Nonenzymatic interactions between proteinases and the cell surface: novel roles in normal and malignant cell physiology. Adv. Cancer Res. 2000; 78:103–157. [PubMed: 10547669]

Miyamoto K, Khosrof S, Bursell SE, Moromizato Y, Aiello LP, Ogura Y, et al. Vascular endothelial growth factor (VEGF)induced retinal vascular permeability is mediated by intercellular adhesion molecule1 (ICAM1). Am. J. Pathol. 2000; 156:1733–1739. [PubMed: 10793084]

Morello CM. Etiology and natural history of diabetic retinopathy: an overview. Am. J. Health Syst. Pharm. 2007; 64 suppl. 12:S3–S7. [PubMed: 17720892]

Mori K, Duh E, Gehlbach P, Ando A, Takahashi K, Pearlman J, et al. Pigment epitheliumderived factor inhibits retinal and choroidal neovascularization. J. Cell Physiol. 2001; 188:253–263. [PubMed: 11424092]

Mori K, Gehlbach P, Ando A, McVey D, Wei L, Campochiaro PA. Regression of ocular neovascularization in response to increased expression of pigment epitheliumderived factor. Invest. Ophthalmol. Vis. Sci. 2002; 43:2428–2434. [PubMed: 12091447]

Morita M, Ohneda O, Yamashita T, Takahashi S, Suzuki N, Nakajima O, et al. HLF/HIF2alpha is a key factor in retinopathy of prematurity in association with erythropoietin. EMBO J. 2003; 22:1134–1146. [PubMed: 12606578]

Moussad EE, Brigstock DR. Connective tissue growth factor. What's in a name. Mol. Genet. Metab. 2000; 71:276–292. [PubMed: 11001822]

Mukherjee PK, Chawla A, Loayza MS, Bazan NG. Docosanoids are multifunctional regulators of neural cell integrity and fate: significance in aging and disease. Prostaglandins Leukot. Essent. Fatty Acids. 2007; 77:233–238. [PubMed: 18060755]

Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and agerelated macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. FASEB J. 2000; 14:835–846. [PubMed: 10783137]

Murata T, Nakagawa K, Khalil A, Ishibashi T, Inomata H, Sueishi K. The temporal and spatial vascular endothelial growth factor expression in retinal vasculogenesis of rat neonates. Lab. Invest. 1996; 74:68–77. [PubMed: 8569199]

Murugeswari P, Shukla D, Rajendran A, Kim R, Namperumalsamy P, Muthukkaruppan V. Proinflammatory cytokines and angiogenic and antiangiogenic factors in vitreous of patients with proliferative diabetic retinopathy and eales' disease. Retina. 2008; 28:817–824. [PubMed: 18536597]

Naduk-Kik J, Hrabec E. The role of matrix metalloproteinases in the pathogenesis of diabetes mellitus and progression of diabetes retinopathy. Postepy Hig. Med. Dosw. 2008; 62:442–450.

Nagineni CN, Samuel W, Nagineni S, Pardhasaradhi K, Wiggert B, Detrick B, et al. Transforming growth factorbeta induces expression of vascular endothelial growth factor in human retinal pigment epithelial cells: involvement of mitogenactivated protein kinases. J. Cell Physiol. 2003; 197:453–462. [PubMed: 14566975]

Nakai K, Fainaru O, Bazinet L, Pakneshan P, Benny O, Pravda E, et al. Dendritic cells augment choroidal neovascularization. Invest. Ophthalmol. Vis. Sci. 2008; 49:3666–3670. [PubMed: 18408184]

Nakamura S, Iwasaki N, Funatsu H, Kitano S, Iwamoto Y. Impact of variants in the VEGF gene on progression of proliferative diabetic retinopathy. Graefes Arch. Clin. Exp. Ophthalmol. 2009; 247:21–26. [PubMed: 18709380]

Nambu H, Nambu R, Oshima Y, Hackett SF, Okoye G, Wiegand S, et al. Angiopoietin 1 inhibits ocular neovascularization and breakdown of the blood retinal barrier. Gene Ther. 2004; 11:865–873. [PubMed: 15042118]

Nambu H, Umeda N, Kachi S, Oshima Y, Akiyama H, Nambu R, et al. Angiopoietin 1 prevents retinal detachment in an aggressive model of proliferative retinopathy, but has no effect on established neovascularization. J. Cell Physiol. 2005; 204:227–235. [PubMed: 15648096]

Nozaki M, Raisler BJ, Sakurai E, Sarma JV, Barnum SR, Lambris JD, et al. Drusen complement components C3a and C5a promote choroidal neovascularization. Proc. Natl. Acad. Sci. USA. 2006; 103:2328–2333. [PubMed: 16452172]

Ogata N, Matsuoka M, Matsuyama K, Shima C, Tajika A, Nishiyama T, et al. Plasma concentration of pigment epitheliumderived factor in patients with diabetic retinopathy. J. Clin. Endocrinol. Metab. 2007; 92:1176–1179. [PubMed: 17213275]

Ogata N, Nishikawa M, Nishimura T, Mitsuma Y, Matsumura M. Unbalanced vitreous levels of pigment epithe-liumderived factor and vascular endothelial growth factor in diabetic retinopathy. Am. J. Ophthalmol. 2002a; 134:348–353. [PubMed: 12208245]

Ogata N, Wada M, Otsuji T, Jo N, TombranTink J, Matsumura M. Expression of pigment epitheliumderived factor in normal adult rat eye and experimental choroidal neovascularization. Invest. Ophthalmol. Vis. Sci. 2002b; 43:1168–1175. [PubMed: 11923262]

Ohlmann AV, Ohlmann A, WelgeLussen U, May CA. Localization of collagen XVIII and endostatin in the human eye. Curr. Eye Res. 2005; 30:27–34. [PubMed: 15875362]

Ohno-Matsui K, Morita I, Tombran-Tink J, Mrazek D, Onodera M, Uetama T, et al. Novel mechanism for agerelated macular degeneration: an equilibrium shift between the angiogenesis factors VEGF and PEDF. J. Cell Physiol. 2001; 189:323–333. [PubMed: 11748590]

Ohno-Matsui K, Yoshida T, Uetama T, Mochizuki M, Morita I. Vascular endothelial growth factor upregulates pigment epitheliumderived factor expression via VEGFR1 in human retinal pigment epithelial cells. Biochem. Biophys. Res. Commun. 2003; 303:962–967. [PubMed: 12670505]

Oka C, Tsujimoto R, Kajikawa M, KoshibaTakeuchi K, Ina J, YanoM, et al. HtrA1 serine protease inhibits signaling mediated by Tgfbeta family proteins. Development. 2004; 131:1041–1053. [PubMed: 14973287]

O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Cao Y, et al. Angiostatin: a circulating endothelial cell inhibitor that suppresses angiogenesis and tumor growth. Cold Spr. Harb. Symp. Quant. Biol. 1994a; 59:471–482.

O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, et al. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell. 1994b; 79:315–328.

O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell. 1997; 88:277–285. [PubMed: 9008168]

O'Reilly MS, Wiederschain D, StetlerStevenson WG, Folkman J, Moses MA. Regulation of angiostatin production by matrix metalloproteinase2 in a model of concomitant resistance. J. Biol. Chem. 1999; 274:29568–295671. [PubMed: 10506224]

Ortego J, Escribano J, Becerra SP, CocaPrados M. Gene expression of the neurotrophic pigment epitheliumderived factor in the human ciliary epithelium. Synthesis and secretion into the aqueous humor. Invest. Ophthalmol. Vis. Sci. 1996; 37:2759–2767. [PubMed: 8977492]

Papapetropoulos A, Garcia-Cardena G, Madri JA, Sessa WC. Nitric oxide production contributes to the angiogenic properties of vascular endothelial growth factor in human endothelial cells. J. Clin. Invest. 1997; 100:3131–3139. [PubMed: 9399960]

Park KW, Morrison CM, Sorensen LK, Jones CA, Rao Y, Chien CB, et al. Robo4 is a vascularspecific receptor that inhibits endothelial migration. Dev. Biol. 2003; 261:251–267. [PubMed: 12941633]

Patel JI, Hykin PG, Gregor ZJ, Boulton M, Cree IA. Angiopoietin concentrations in diabetic retinopathy. Br. J. Ophthalmol. 2005; 89:480–483. [PubMed: 15774928]

Patel M, Chan CC. Immunopathological aspects of age-related macular degeneration. Semin Immunopathol . 2008; 30:97–110. [PubMed: 18299834]

Patel JI, TombranTink J, Hykin PG, Gregor ZJ, Cree IA. Vitreous and aqueous concentrations of proangiogenic, antiangiogenic factors and other cytokines in diabetic retinopathy patients with macular edema: Implications for structural differences in macular profiles. Exp. Eye Res. 2006; 82:798–806. [PubMed: 16324700]

Patel S, Rowe MJ, Winters SA, Ohls RK. Elevated erythropoietin mRNA and protein concentrations in the developing human eye. Pediatr. Res. 2008; 63:394–397. [PubMed: 18356745]

Paulus YM, Gariano RF. Diabetic retinopathy: a growing concern in an aging population. Geriatrics. 2009; 64:16–20. [PubMed: 19256582]

Pelikanova T. Pathogenesis of diabetic retinopathy. Vnitr. Lek. 2007; 53:498–505. [PubMed: 17642432]

Penfold PL, Provis JM, Billson FA. Agerelated macular degeneration: ultrastructural studies of the relationship of leucocytes to angiogenesis. Graefes Arch. Clin. Exp. Ophthalmol. 1987; 225:70–76. [PubMed: 2436980]

Penn JS, Madan A, Caldwell RB, Bartoli M, Caldwell RW, Hartnett ME. Vascular endothelial growth factor in eye disease. Prog. Retin Eye Res. 2008; 27:331–371. [PubMed: 18653375]

Peters KG. Vascular endothelial growth factor and the angiopoietins: working together to build a better blood vessel. Circ. Res. 1998; 83:342–343. [PubMed: 9710128]

Pierce EA, Avery RL, Foley ED, Aiello LP, Smith LE. Vascular endothelial growth factor/vascular permeability factor expression in a mouse model of retinal neovascularization. Proc. Natl. Acad. Sci. USA. 1995; 92:905–909. [PubMed: 7846076]

Pierce EA, Foley ED, Smith LE. Regulation of vascular endothelial growth factor by oxygen in a model of retinopathy of prematurity. Arch. Ophthalmol. 1996; 114:1219–1228. [PubMed: 8859081]

Plantner JJ, Jiang C, Smine A. Increase in interphotoreceptor matrix gelatinase A (MMP-2) associated with age-related macular degeneration. Exp. Eye Res. 1998; 67:637–645. [PubMed: 9990329]

Poulaki V, Joussen AM, Mitsiades N, Mitsiades CS, Iliaki EF, Adamis AP. Insulinlike growth factorI plays a pathogenetic role in diabetic retinopathy. Am. J. Pathol. 2004; 165:457–469. [PubMed: 15277220]

Presta M, Tiberio L, Rusnati M, Dell'Era P, Ragnotti G. Basic fibroblast growth factor requires a longlasting activation of protein kinase C to induce cell proliferation in transformed fetal bovine aortic endothelial cells. Cell Regul. 1991; 2:719–726. [PubMed: 1742342]

Presta M, Dell'Era P, Mitola S, Moroni E, Ronca R, Rusnati M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev. 2005; 16:159–178. [PubMed: 15863032]

Presta M, Andres G, Leali D, Dell'era P, Ronca R. Inflammatory cells and chemokines sustain FGF2induced angiogenesis. Eur. Cytokine Netw. 2009; 20:39–50. [PubMed: 19541589]

Prosser BE, Johnson S, Roversi P, Herbert AP, Blaum BS, Tyrrell J, et al. Structural basis for complement factor H linked agerelated macular degeneration. J. Exp. Med. 2007; 204:2277–2283. [PubMed: 17893204]

Ray D, Mishra M, Ralph S, Read I, Davies R, Brenchley P. Association of the VEGF gene with proliferative diabetic retinopathy but not proteinuria in diabetes. Diabetes. 2004; 53:861–864. [PubMed: 14988276]

Renno RZ, Youssri AI, Michaud N, Gragoudas ES, Miller JW. Expression of pigment epitheliumderived factor in experimental choroidal neovascularization. Invest. Ophthalmol. Vis . Sci. 2002; 43:1574–1580. [PubMed: 11980876]

Roberts WG, Palade GE. Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. J. Cell Sci. 1995; 108:2369–2379. [PubMed: 7673356]

Rosenthal R, Malek G, Salomon N, PeillMeininghaus M, Coeppicus L, Wohlleben H, et al. The fibroblast growth factor receptors, FGFR1 and FGFR2, mediate two independent signalling pathways in human retinal pigment epithelial cells. Biochem. Biophys. Res. Commun. 2005; 337:241–247. [PubMed: 16188231]

Ross RJ, Bojanowski CM, Wang JJ, Chew EY, Rochtchina E, Ferris FL3rd, et al. The LOC387715 polymorphism and agerelated macular degeneration: replication in three casecontrol samples. Invest. Ophthalmol. Vis. Sci. 2007; 48:1128–1132. [PubMed: 17325155]

Rousseau B, Dubayle D, Sennlaub F, Jeanny JC, Costet P, Bikfalvi A, et al. Neural and angiogenic defects in eyes of transgenic mice expressing a dominantnegative FGF receptor in the pigmented cells. Exp. Eye Res. 2000; 71:395–404. [PubMed: 10995560]

Ruberte J, Ayuso E, Navarro M, Carretero A, Nacher V, Haurigot V, et al. Increased ocular levels of IGF1 in transgenic mice lead to diabeteslike eye disease. J. Clin. Invest. 2004; 113:1149–1157. [PubMed: 15085194]

Sack RA, Beaton AR, Sathe S. Diurnal variations in angiostatin in human tear fluid: a possible role in prevention of corneal neovascularization. Curr. Eye Res. 1999; 18:186–193. [PubMed: 10342373]

Saishin Y, Silva RL, Kachi S, Aslam S, Gong YY, Lai H, et al. Periocular gene transfer of pigment epithelium-derived factor inhibits choroidal neovascularization in a human-sized eye. Hum. Gene. Ther. 2005; 16:473–478. [PubMed: 15871678]

Sakurai E, Anand A, Ambati BK, van Rooijen N, Ambati J. Macrophage depletion inhibits experimental choroidal neovascularization. Invest. Ophthalmol. Vis. Sci. 2003a; 44:3578–3585. [PubMed: 12882810]

Sakurai E, Taguchi H, Anand A, Ambati BK, Gragoudas ES, Miller JW, et al. Targeted disruption of the CD18 or ICAM1 gene inhibits choroidal neovascularization. Invest. Ophthalmol. Vis. Sci. 2003b; 44:2743–2749.

Sakurai Y, Ohgimoto K, Kataoka Y, Yoshida N, Shibuya M. Essential role of Flk1 (VEGF receptor 2) tyrosine residue 1173 in vasculogenesis in mice. Proc. Natl. Acad. Sci. USA. 2005; 102:1076–1081. [PubMed: 15644447]

Sall JW, Klisovic DD, O'Dorisio MS, Katz SE. Somatostatin inhibits IGF1 mediated induction of VEGF in human retinal pigment epithelial cells. Exp. Eye Res. 2004; 79:465–476. [PubMed: 15381031]

Sarlos S, Rizkalla B, Moravski CJ, Cao Z, Cooper ME, WilkinsonBerka JL. Retinal angiogenesis is mediated by an interaction between the angiotensin type 2 receptor, VEGF, and angiopoietin. Am. J. Pathol. 2003; 163:879–887. [PubMed: 12937129]

Sato T, Kusaka S, Shimojo H, Fujikado T. Vitreous Levels of Erythropoietin and Vascular Endothelial Growth Factor in Eyes with Retinopathy of Prematurity. Ophthalmology. 2009; 116:1599–1603. [PubMed: 19371954]

Schneider JK, Gardner DK, Cordero L. Use of recombinant human erythropoietin and risk of severe retinopathy in extremely lowbirthweight infants. Pharmacotherapy. 2008; 28:1335–1340. [PubMed: 18956993]

Schwesinger C, Yee C, Rohan RM, Joussen AM, Fernandez A, Meyer TN, et al. Intrachoroidal neovascularization in transgenic mice overexpressing vascular endothelial growth factor in the retinal pigment epithelium. Am. J. Pathol. 2001; 158:1161–1172. [PubMed: 11238064]

Sears JE, Hoppe G, Ebrahem Q, AnandApte B. Prolyl hydroxylase inhibition during hyperoxia prevents oxygeninduced retinopathy. Proc. Natl. Acad. Sci. USA. 2008; 105:19898–19903. [PubMed: 19057008]

Seddon JM, Gensler G, Milton RC, Klein ML, Rifai N. Association between Creactive protein and agerelated macular degeneration. JAMA. 2004; 291:704–710. [PubMed: 14871913]

Sepp NT, Li LJ, Lee KH, Brown EJ, Caughman SW, Lawley TJ, et al. Basic fibroblast growth factor increases expression of the alpha v beta 3 integrin complex on human microvascular endothelial cells. J. Invest. Dermatol. 1994; 103:295–299. [PubMed: 8077694]

She H, Nakazawa T, Matsubara A, Hisatomi T, Young TA, Michaud N, et al. Reduced photoreceptor damage after photodynamic therapy through blockade of nitric oxide synthase in a model of choroidal neovascularization. Invest. Ophthalmol. Vis. Sci. 2007; 48:2268–2277. [PubMed: 17460290]

Shi XH, He SZ, Zhao SH. Expression and signification of pigment epitheliumderived factor in experimental choroidal neovascularization of rat. Zhonghua Yan Ke Za Zhi. 2004; 40:404–408. [PubMed: 15312607]

Shibuya M, ClaessonWelsh L. Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. Exp. Cell Res. 2006; 312:549–560. [PubMed: 16336962]

Shih SC, Ju M, Liu N, Smith LE. Selective stimulation of VEGFR1 prevents oxygeninduced retinal vascular degeneration in retinopathy of prematurity. J. Clin. Invest. 2003; 112:50–57. [PubMed: 12840058]

Shing Y, Folkman J, Sullivan R, Butterfield C, Murray J, Klagsbrun M. Heparin affinity: purification of a tumorderived capillary endothelial cell growth factor. Science. 1984; 223:1296–1299. [PubMed: 6199844]

Shing Y, Folkman J, Haudenschild C, Lund D, Crum R, Klagsbrun M. Angiogenesis is stimulated by a tumorderived endothelial cell growth factor. J. Cell Biochem. 1985; 29:275–287. [PubMed: 2418039]

Shono T, Kanetake H, Kanda S. The role of mitogenactivated protein kinase activation within focal adhesions in chemotaxis toward FGF2 by murine brain capillary endothelial cells. Exp. Cell Res. 2001; 264:275–283. [PubMed: 11262184]

Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxiainitiated angiogenesis. Nature. 1992; 359:843–845. [PubMed: 1279431]

Silva KC, Pinto CC, Biswas SK, de Faria JB, de Faria JM. Hypertension increases retinal inflammation in experimental diabetes: a possible mechanism for aggravation of diabetic retinopathy by hypertension. Curr. Eye Res. 2007; 32:533–541. [PubMed: 17612969]

Simo R, Carrasco E, Garcia-Ramirez M, Hernandez C. Angiogenic and antiangiogenic factors in proliferative diabetic retinopathy. Curr. Diabetes Rev. 2006; 2:71–98. [PubMed: 18220619]

Singh N, Amin S, Richter E, Rashid S, Scoglietti V, Jani PD, et al. Flt-1 intraceptors inhibit hypoxia-induced VEGF expression *in vitro* and corneal neovascularization *in vivo*. Invest. Ophthalmol. Vis. Sci. 2005a; 46:1647–1652. [PubMed: 15851564]

Singh N, Macnamara E, Rashid S, Ambati J, Kontos CD, Higgins E, et al. Systemic soluble Tie2 expression inhibits and regresses corneal neovascularization. Biochem. Biophys. Res. Commun. 2005b; 332:194–199. [PubMed: 15896317]

Singh N, Jani PD, Suthar T, Amin S, Ambati BK. Flt-1 intraceptor induces the unfolded protein response, apoptotic factors, and regression of murine injury induced corneal neovascularization. Invest. Ophthalmol. Vis. Sci. 2006; 47:4787–4793. [PubMed: 17065489]

Singh N, Higgins E, Amin S, Jani P, Richter E, Patel A, et al. Unique homologous siRNA blocks hypoxia-induced VEGF upregulation in human corneal cells and inhibits and regresses murine corneal neovascularization. Cornea. 2007; 26:65–72. [PubMed: 17198016]

Skopinski P, Rogala E, Duda-Krol B, Lipinska A, Sommer E, Chorostowska-Wynimko J, et al. Increased interleukin-18 content and angiogenic activity of sera from diabetic (Type 2) patients with background retinopathy. J. Diabet. Complications. 2005; 19:335–338.

Smith LE. IGF-1 and retinopathy of prematurity in the preterm infant. Biol. Neonate. 2005; 88:237–244. [PubMed: 16210846]

Smith LE. Through the eyes of a child: understanding retinopathy through ROP the Friedenwald lecture. Invest. Ophthalmol. Vis. Sci. 2008; 49:5177–5182. [PubMed: 18708611]

Smith LE, Kopchick JJ, Chen W, Knapp J, Kinose F, Daley D, et al. Essential role of growth hormone in ischemia-induced retinal neovascularization. Science. 1997; 276:1706–1709. [PubMed: 9180082]

Smith LE, Shen W, Perruzzi C, Soker S, Kinose F, Xu X, et al. Regulation of vascular endothelial growth factor-dependent retinal neovascularization by insulin-like growth factor-1 receptor. Nat. Med. 1999; 5:1390–1395. [PubMed: 10581081]

Soubrane G, Jerdan J, Karpouzas I, Fayein NA, Glaser B, Coscas G, et al. Binding of basic fibroblast growth factor to normal and neovascularized rabbit cornea. Invest. Ophthalmol. Vis. Sci. 1990; 31:323–333. [PubMed: 1689281]

Soubrane G, Cohen SY, Delayre T, Tassin J, Hartmann MP, Coscas GJ, et al. Basic fibroblast growth factor experimentally induced choroidal angiogenesis in the minipig. Curr. Eye Res. 1994; 13:183–195. [PubMed: 7514965]

Spencer KL, Hauser MA, Olson LM, Schmidt S, Scott WK, Gallins P, et al. Protective effect of complement factor B and complement component 2 variants in agerelated macular degeneration. Hum. Mol. Genet. 2007; 16:1986–1992. [PubMed: 17576744]

Stokes CL, Rupnick MA, Williams SK, Lauffenburger DA. Chemotaxis of human microvessel endothelial cells in response to acidic fibroblast growth factor. Lab. Invest. 1990; 63:657–668. [PubMed: 1700197]

Suchting S, Heal P, Tahtis K, Stewart LM, Bicknell R. Soluble Robo4 receptor inhibits *in vivo* angiogenesis and endothelial cell migration. FASEB J. 2005; 19:121–123. [PubMed: 15486058]

Suganthalakshmi B, Anand R, Kim R, Mahalakshmi R, Karthikprakash S, Namperumalsamy P, et al. Association of VEGF and eNOS gene polymorphisms in type 2 diabetic retinopathy. Mol. Vis. 2006; 12:336–341. [PubMed: 16636650]

Sugihara T, Wadhwa R, Kaul SC, Mitsui Y. A novel alternatively spliced form of murine vascular endothelial growth factor, VEGF 115. J. Biol. Chem. 1998; 273:3033–3038. [PubMed: 9446618]

Suk KK, Dunbar JA, Liu A, Daher NS, Leng CK, Leng JK, et al. Human recombinant erythropoietin and the incidence of retinopathy of prematurity: a multiple regression model. J. AAPOS. 2008; 12:233–238. [PubMed: 18589385]

Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, et al. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell. 1996; 87:1171–1180. [PubMed: 8980224]

Suri C, McClain J, Thurston G, McDonald DM, Zhou H, Oldmixon EH, et al. Increased vascularization in mice over-expressing angiopoietin-1. Science. 1998; 282:468–471. [PubMed: 9774272]

Suzuma K, Takagi H, Otani A, Oh H, Honda Y. Expression of thrombospondin-1 in ischemia-induced retinal neovascularization. Am. J. Pathol. 1999; 154:343–354. [PubMed: 10027393]

Swaroop A, Branham KE, Chen W, Abecasis G. Genetic susceptibility to age-related macular degeneration: a paradigm for dissecting complex disease traits. Hum. Mol. Genet. 2007; 16:R174–R182. [PubMed: 17911160]

Takagi H, Watanabe D, Suzuma K, Kurimoto M, Suzuma I, Ohashi H, et al. Novel role of erythropoietin in proliferative diabetic retinopathy. Diabetes Res. Clin. Pract. 2007; 77:S62–S64. [PubMed: 17481772]

Takahashi H, Shibuya M. The vascular endothelial growth factor (VEGF)/VEGF receptor system and its role under physiological and pathological conditions. Clin. Sci. 2005; 109:227–241. [PubMed: 16104843]

Tang NP, Zhou B, Wang B, Yu RB. HTRA1 promoter polymorphism and risk of age-related macular degeneration: a meta-analysis. Ann. Epidemiol. 2009; 19:740–745. [PubMed: 19375943]

Tawfik A, Sanders T, Kahook K, Akeel S, Elmarakby A, Al-Shabrawey M. Suppression of retinal peroxisome proliferator-activated receptor gamma in experimental diabetes and oxygen-induced retinopathy: role of NADPH oxidase. Invest. Ophthalmol. Vis. Sci. 2009; 50:878–884. [PubMed: 18806296]

Teng Y, Cui H, Yang M, Song H, Zhang Q, Su Y, et al. Protective effect of puerarin on diabetic retinopathy in rats. Mol. Biol. Rep. 2009; 36:1129–1133. [PubMed: 18587665]

Terry TL. Fibroblastic overgrowth of persistent Tunica Vasculosa Lentis in infants born prematurely: II. Report of cases clinical aspects. Trans. Am. Ophthalmol. Soc. 1942; 40:262–284. [PubMed: 16693285]

Thakkinstian A, Han P, McEvoy M, Smith W, Hoh J, Magnusson K, et al. Systematic review and meta-analysis of the association between complement factor H Y402H polymorphisms and age-related macular degeneration. Hum. Mol. Genet. 2006; 15:2784–2790. [PubMed: 16905558]

Tong JP, Shen Y, Chan WM, Lin SC, Peng ZP. Vascular endothelial growth factor and pigment epithelium-derived factor in aqueous humor of patients with choroidal neovascularization. Zhejiang Da Xue Xue Bao Yi Xue Ban. 2006; 35:311–314. [PubMed: 16764035]

Triebel J, Huefner M, Ramadori G. Investigation of prolactin-related vasoinhibin in sera from patients with diabetic retinopathy. Eur. J. Endocrinol. 2009; 161:345–353. [PubMed: 19477896]

Tsutsumi C, Sonoda KH, Egashira K, Qiao H, Hisatomi T, Nakao S, et al. The critical role of ocular-infiltrating macrophages in the development of choroidal neovascularization. J. Leukoc. Biol. 2003; 74:25–32. [PubMed: 12832439]

Tuo J, Smith BC, Bojanowski CM, Meleth AD, Gery I, Csaky KG, et al. The involvement of sequence variation and expression of CX3CR1 in the pathogenesis of age-related macular degeneration. FASEB J. 2004; 18:1297–1299. [PubMed: 15208270]

Twining SS, Wilson PM, Ngamkitidechakul C. Extrahepatic synthesis of plasminogen in the human cornea is up-regulated by interleukins-1alpha and - 1beta. Biochem. J. 1999; 339:705–712. [PubMed: 10215610]

Uhlmann S, Friedrichs U, Eichler W, Hoffmann S, Wiedemann P. Direct measurement of VEGF-induced nitric oxide production by choroidal endothelial cells. Microvasc. Res. 2001; 62:179–189. [PubMed: 11516247]

Underwood PA, Bean PA, Gamble JR. Rate of endothelial expansion is controlled by cell:cell adhesion. Int. J. Biochem. Cell. Biol. 2002; 34:55–69. [PubMed: 11733185]

Uthra S, Raman R, Mukesh BN, Rajkumar SA, Kumari RP, Agarwal S, et al. Diabetic retinopathy and IGF-1 gene polymorphic cytosineadenine repeats in a Southern Indian cohort. Ophthalmic. Res. 2007; 39:294–299. [PubMed: 17851271]

Uthra S, Raman R, Mukesh BN, Rajkumar SA, Padmaja KR, Paul PG, et al. Association of VEGF gene polymorphisms with diabetic retinopathy in a south Indian cohort. Ophthalmic Genet. 2008; 29:11–15. [PubMed: 18363167]

Vanhaesebrouck S, Daniels H, Moons L, Vanhole C, Carmeliet P, De Zegher F. Oxygen-induced retinopathy in mice: amplification by neonatal IGF-I deficit and attenuation by IGF-I administration. Pediatr. Res. 2009; 65:307–310. [PubMed: 19092722]

Villegas-Becerril E, Gonzalez-Fernandez R, Perula-Torres L, Gallardo-Galera JM. IGF-I, VEGF and bFGF as predictive factors for the onset of retinopathy of prematurity (ROP). Arch. Soc. Esp. Oftalmol. 2006; 81:641–646. [PubMed: 17136637]

Wakabayashi Y, Usui Y, Okunuki Y, Takeuchi M, Kezuka T, Iwasaki T, et al. Increased levels of monokine induced by interferon-gamma (Mig) in the vitreous of patients with diabetic retinopathy. Diabetic Med. 2008; 25:875–877. [PubMed: 18644076]

Wang FH, Sun XD, Zhang X, Xu X, Zhu Q, Huang JN, et al. Role of pigment epithelium-derived factor on proliferation and migration of choroidal capillary endothelium induced by vascular endothelial growth factor in vitro. Chin. Med. J. 2007; 120:1534–1538. [PubMed: 17908464]

Wang ZY, Shen LJ, Tu L, Hu DN, Liu GY, Zhou ZL, et al. Erythropoietin protects retinal pigment epithelial cells from oxidative damage. Free Radic. Biol. Med. 2009; 46:1032–1041. [PubMed: 19136057]

Watanabe D. Erythropoietin as a retinal angiogenic factor in proliferative diabetic retinopathy. Nippon Ganka Gakkai Zasshi. 2007; 111:892–898. [PubMed: 18051819]

Watanabe D, Suzuma K, Matsui S, Kurimoto M, Kiryu J, Kita M, et al. Erythropoietin as a retinal angiogenic factor in proliferative diabetic retinopathy. N. Engl. J. Med. 2005a; 353:782–792. [PubMed: 16120858]

Watanabe D, Suzuma K, Suzuma I, Ohashi H, Ojima T, Kurimoto M, et al. Vitreous levels of angiopoietin 2 and vascular endothelial growth factor in patients with proliferative diabetic retinopathy. Am. J. Ophthalmol. 2005b; 139:476–481. [PubMed: 15767056]

Watanabe D, Takagi H, Suzuma K, Oh H, Ohashi H, Honda Y, et al. Expression of connective tissue growth factor and its potential role in choroidal neovascularization. Retina. 2005c; 25:911–918. [PubMed: 16205572]

Werdich XQ, McCollum GW, Rajaratnam VS, Penn JS. Variable oxygen and retinal VEGF levels: correlation with incidence and severity of pathology in a rat model of oxygen-induced retinopathy. Exp. Eye Res. 2004; 79:623–630. [PubMed: 15500821]

Wilkinson-Berka JL. Vasoactive factors and diabetic retinopathy: vascular endothelial growth factor, cycoloxygenase-2 and nitric oxide. Curr. Pharm. Des. 2004; 10:3331–3348. [PubMed: 15544519]

Wilkinson-Berka JL. Angiotensin and diabetic retinopathy. Int. J. Biochem. Cell Biol. 2006; 38:752–765. [PubMed: 16165393]

Wilkinson-Berka JL, Fletcher EL. Angiotensin and bradykinin: targets for the treatment of vascular and neuro-glial pathology in diabetic retinopathy. Curr. Pharm. Des. 2004; 10:3313–3330. [PubMed: 15544518]

Wilkinson-Berka JL, Babic S, De Gooyer T, Stitt AW, Jaworski K, Ong LG, et al. Inhibition of platelet-derived growth factor promotes pericyte loss and angiogenesis in ischemic retinopathy. Am. J. Pathol. 2004; 164:1263–1273. [PubMed: 15039215]

Wilkinson-Berka JL, Jones D, Taylor G, Jaworski K, Kelly DJ, Ludbrook SB, et al. SB-267268, a nonpeptidic antagonist of alpha(v)beta3 and alpha(v)beta5 integrins, reduces angiogenesis and VEGF expression in a mouse model of retinopathy of prematurity. Invest. Ophthalmol. Vis. Sci. 2006; 47:1600–1605. [PubMed: 16565398]

Witzenbichler B, Maisonpierre PC, Jones P, Yancopoulos GD, Isner JM. Chemotactic properties of angiopoietin-1 and −2, ligands for the endothelial- specific receptor tyrosine kinase Tie2. J. Biol. Chem. 1998; 273:18514–18521. [PubMed: 9660821]

Woessner JF Jr. The family of matrix metalloproteinases. Ann. N. Y. Acad. Sci. 1994; 732:11–21. [PubMed: 7978784]

Wu WC, Kao YH, Hu PS, Chen JH. Geldanamycin, a HSP90 inhibitor, attenuates the hypoxia-induced vascular endothelial growth factor expression in retinal pigment epithelium cells in vitro. Exp. Eye Res. 2007; 85:721–731. [PubMed: 17870069]

Xie B, Shen J, Dong A, Swaim M, Hackett SF, Wyder L, et al. An Adam 15 amplification loop promotes vascular endothelial growth factor-induced ocular neovascularization. FASEB J. 2008; 22:2775–2783. [PubMed: 18381816]

Yamagishi S, Nakamura K, Imaizumi T. Advanced glycation end products (AGEs) and diabetic vascular complications. Curr. Diabetes Rev. 2005; 1:93–106. [PubMed: 18220586]

Yamagishi S, Matsui T, Nakamura K, Inoue H, Takeuchi M, Ueda S, et al. Olmesartan blocks advanced glycation end products (AGEs)-induced angiogenesis in vitro by suppressing receptor for AGEs (RAGE) expression. Microvasc. Res. 2008; 75:130–134. [PubMed: 17560613]

Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, et al. Holash 2000. Vascular-specific growth factors and blood vessel formation. Nature. 2000; 407:242–248. [PubMed: 11001067]

Yang Z, Stratton C, Francis PJ, Kleinman ME, Tan PL, Gibbs D, et al. Toll-like receptor 3 and geographic atrophy in age-related macular degeneration. N. Engl. J. Med. 2008; 359:1456–1463. [PubMed: 18753640]

Yates JR, Sepp T, Matharu BK, Khan JC, Thurlby DA, Shahid H, et al. Complement C3 variant and the risk of age-related macular degeneration. N. Engl. J. Med. 2007; 357:553–561. [PubMed: 17634448]

Yaylali V, Ohta T, Kaufman SC, Maitchouk DY, Beuerman RW. In vivo confocal imaging of corneal neovascularization. Cornea. 1998; 17:646–653. [PubMed: 9820946]

Ye HQ, Azar DT. Expression of gelatinases A and B, and TIMPs 1 and 2 during corneal wound healing. Invest. Ophthalmol. Vis. Sci. 1998; 39:913–921. [PubMed: 9579471]

Ye HQ, Maeda M, Yu FS, Azar DT. Differential expression of MT1-MMP (MMP-14) and collagenase III (MMP-13) genes in normal and wounded rat corneas. Invest. Ophthalmol. Vis. Sci. 2000; 41:2894–2899. [PubMed: 10967042]

Yokoi M, Yamagishi S, Saito A, Yoshida Y, Matsui T, Saito W, et al. Positive association of pigment epithelium-derived factor with total antioxidant capacity in the vitreous fluid of patients with proliferative diabetic retinopathy. Br. J. Ophthalmol. 2007; 91:885–887. [PubMed: 17301120]

Zhou J, Pham L, Zhang N, He S, Gamulescu MA, Spee C, et al. Neutrophils promote experimental choroidal neovascularization. Mol. Vis. 2005; 11:414–424. [PubMed: 15988410]

Zubilewicz A, Hecquet C, Jeanny JC, Soubrane G, Courtois Y, Mascarelli. Two distinct signalling pathways are involved in FGF2-stimulated proliferation of choriocapillary endothelial cells: a comparative study with VEGF. Oncogene. 2001; 20:1403–1413. [PubMed: 11313884]

Table 1

Clinical conditions associated with corneal neovascularization.

Infectious	**Trauma**
Herpes simplex keratitis	Alkali burns
Herpes zoster keratitis	Contact lens
Syphilis	Ulceration,
Pseudomonas	
Chamydia trachomatis	**Degenerative**
Candidiasis	Terrien marginal degeneration
Fusarium	Pterygium
Aspergillosis	Aniridia
Onchocerciasis	
	Iatrogenic
Inflammatory	
Graft rejection	
Acne rosacea	
Stevens-Johnson syndrome	
GVHD	
Pemphigoid	
Atopic conjunctivitis	

Table 2

Angiogenic and anti-angiogenic factors in corneal neovascularization

Angiogenic molecules	Anti-angiogenic molecules
VEGF	PEDF
FGF	Endostatin
PlGF	Angiostatin
TGF-α, TGF-β	PRL
IGF	TIMPs
PDGF	TSP-1. TSP-2
MMPs	IL-4, −12, −13, −18
HGF/SF	Arresten
TNF- α	Canstatin
CTGF	Tumstatin
IL-1, −8	TNF-α
MCP-1	MMPs
Leptin	IFN-γ
Integrins (αV β3)	
Angiogenin	
TXA-2, COX-2	
NO	
PAF	

Table 3

Factors mediating retinal angiogenesis

Factors	Mechanism
VEGF	increases permeability
HIF	upregulator of VEGF
EPO	erythropoeisis, angiogenesis and endothelial cell proliferation, antioxidative and neuroprotective properties
IGF	promotes growth of vasculature by VEGF
Omega 3-PUFA's	maternally derivded amino acids that aid increase vessel regrowth after injury and neuroprotective
PEDF	inhibits ocular neovascularization and VEGF induced migration and endothelial cell proliferation
FGF	synergistic with VEGF and increase macular EC proliferation and sprout formation
CTGF	extracellular matrix production, endothelial cell adhesion, migration and survival
TGF-beta	controls extracellular matrix production, anti-proliferative in normal cells and induces apoptosis
Ang1	stabilizes vessels, inhibitory to I-CAM, V-CAM, and E-selectin
Ang2	causes vascular hyperpermeability and leakiness
Tie2	stabilizes vessels
NO	pro-angiogenic via increase in VEGF
I-CAMs	adhesion molecules that aid VEGF chemotactic ability
MMPs	involved in the degradation and remodeling of ECM to facilitate new vasculature
TIMP	counteracts the actions of MMP, inhibits VEGF and FGF induced chemotaxis and angiogenesis
MCP-1	macrophage recruitment
Angiotensin II	increases leukocytosis
CRP	associated with increased CNV
CHF	negative regulator of complement and CRP- associated with decreased CNV
Complement factor B	activator of complement
Complement component 2	activator of complement
HTRA1	apoptosis and degradation of ECM molecules, inhibitory to TGF-beta
TLR3	triggers inflammatory cascade leading to apoptosis