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Negative regulators of angiogenesis: Important targets for treatment of exudative AMD

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Abstract

Angiogenesis contributes to pathogenesis of many diseases including exudative age-related macular degeneration (AMD). It is normally kept in check by a tightly balanced production of pro- and anti-angiogenic factors. The up-regulation of the proangiogenic factor, vascular endothelial growth factor, is intimately linked to the pathogenesis of exudative AMD, and its antagonism has been effectively targeted for treatment. However, very little is known about potential changes in expression of antiangiogenic factors and the role they play in choroidal vascular homeostasis and neovascularization associated with AMD. Here we will discuss the important role of thrombospondins and pigment epithelium derived factor, two major endogenous inhibitors of angiogenesis, in retinal and choroidal vascular homeostasis and their potential alterations during AMD and choroidal neovascularization. We will review the cell autonomous function of these proteins in retinal and choroidal vascular cells. We will also discuss the potential targeting of these molecules and use of their mimetic peptides for therapeutic development for exudative AMD.

Keywords

Thrombospondins; PEDF; Angiogenesis; Endothelial cells; Pericytes; Retinal pigment epithelial cells

INTRODUCTION

Age related macular degeneration (AMD) is a major cause of visual impairment in the elderly population worldwide. In 2010, macular degeneration (mainly age-related) was the

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third most common cause of blindness (8.69% of all cases in 2010) [1]. The global occurrence of AMD is expected to increase with nearly a six-fold rise in aging population from 2010 to 2050. Despite the high prevalence of AMD, its etiology remains largely unknown. AMD is characterized by a progressive degeneration of the macula, and severe vision loss. It presents in two major forms, the dry form which is associated with degeneration of retinal pigmented epithelium (RPE) and photoreceptors, and the exudative or wet form which presents the formation of choroidal neovascularization (CNV) [2, 3]. Neovascularization in the exudative AMD is classified to different subtypes including type 1 (sub-RPE), type 2 (sub-retinal), type 3 (intraretinal), and mixed neovascularization [4]. The detailed mechanisms underlying the pathogenesis of CNV is not well understood. Early phase of CNV is associated with altered production of angioregulatory factors, and enhanced migration and proliferation of choroidal endothelial cells (ChEC) in the retina rupturing through the Bruch's membrane. In the active phase, CNV starts to expand, either by remaining beneath the RPE or by entering the sub-retinal space (Figure 1). Ultimately, in the end phase CNV becomes fibrotic and represents disciform scars [5–7]. Diameter and thickness of these disciform scars might be influenced by the degree of RPE and photoreceptor cell degeneration [7].

The increased production of vascular endothelial growth factor (VEGF), a potent proangiogenic factor, is identified as an essential factor in the development and progression of AMD and CNV. Others and we have also proposed that changes in the expression of endogenous inhibitors of angiogenesis, including thrombospondin-1 (TSP1) and pigmented epithelium derived factor (PEDF), make significant contributions to pathogenesis of AMD and CNV, and are major subjects of this review.

The choroidal vasculature plays a crucial role in retinal homeostasis and vision. It functions to dissipate heat and nourish the RPE and outer retinal photoreceptor cells through their fenestrated capillary endothelial cells (EC). Choroidal vascular dysfunction is now recognized as a critical early event in the development and progression of AMD and CNV. The choroid and its vascular system may also be impacted by alterations in inner retinal vasculature as occurs in retinopathy of prematurity and diabetic retinopathy. However, the detailed mechanisms that impact ChEC function and pathogenesis of AMD remains poorly understood, and may be influenced by altered production of many pro- and anti-angiogenic factors including VEGF, PEDF and TSP1, to name a few.

The RPE cells are a major source of ocular angioregulatory proteins, which maintain ocular angiogenesis in check. The impairment of RPE cell function is an early and crucial event in the molecular pathways that lead to clinically relevant AMD changes, and alterations in the angiogenic balance that drives CNV. Neovascularization occurs as a result of altered balanced production of pro- and anti-angiogenic factors, most likely by RPE cells, and may contribute to various eye diseases including diabetic retinopathy and exudative AMD [8–10]. Many studies have reported impaired production of TSP1 and PEDF in these vascular retinopathies [11]. However, the underlying mechanisms, which contribute to these changes remain largely unexplored.

In humans, TSP1 is present at high levels in the vitreous and aqueous humor, and is a major component of the Bruch's membrane [12, 13]. Decreased TSP1 level in the Bruch's membrane and choroidal vessels during AMD is suggested as a potential contributing factor favoring the formation of CNV [12]. PEDF, a glycoprotein (50 kDa), is also an endogenous inhibitor of angiogenesis and it is present in the vitreous at high levels [14]. A number of studies have demonstrated important roles for PEDF in modulation of vascular leakage and angiogenesis in AMD and DR [15, 16]. Patients with the dry form of AMD demonstrate a significant decrease in the PEDF plasma levels. In contrast, patients with wet AMD demonstrate a strong positive correlation between VEGF and PEDF concentrations [17].

Drugs that antagonize VEGF activity are currently the standard of care for treatment of exudative AMD, and effectively delay disease progression in most patients. However, VEGF antagonists significantly improve vision in only 30% of patients, and 20% of treated patients still progress to severe vision loss, becoming legally blind. Combined therapeutic regimens incorporating low-dosage of VEGF antagonists has been suggested as a potential therapy to provide more effective and safer treatment for AMD. Thus, a greater need for development of alternative treatments is highly justified.

Here we will review the physiological role and functional relevance of endogenous inhibitors of angiogenesis, namely TSP1 and PEDF, in ocular vascular homeostasis, and their potential contributions to pathologies of exudative AMD. Furthermore, we propose the potential use of short peptides that mimic the antiangiogenic action of these molecules in the treatment of exudative AMD, alone or in combination with existing therapies.

AMD AND ENDOGENOUS INHIBITORS OF ANGIOGENESIS

Ocular vascular homeostasis

Ocular vascular homeostasis is maintained by a balanced production of positive and negative angioregulatory factors. Different pathological conditions including inflammation, oxidative stress and/or ischemia can disrupt this balance by inducing production of pro-angiogenic factors while suppressing the production of anti-angiogenic factors in damaged cells [18, 19]. The presence of several endogenous inhibitors of angiogenesis including, TSP1, PEDF, endostatin, and angiostatin have been reported in the eye [20, 21]. Some of these anti-angiogenic factors need proteolytic processing for their activation [20]. For example, endostatin has antiangiogenic activity only after proteolytic cleavage [21].

TSP1 and PEDF both inhibit angiogenesis through selective induction of apoptosis of EC involved in formation of new blood vessels, without a significant impact on the EC of existing mature blood vessels [22, 23]. Decreased levels of TSP1, PEDF, and endostatin in Bruch's membrane and choriocapillaris complex has been detected in ocular samples from patients with AMD. Furthermore, high levels of TSP1 and PEDF accumulate in disciform scars, a hallmark of CNV formation [5]. These studies suggest that alterations in production of angiogenesis inhibitors by the RPE cells, and their diminished presence in Bruch's membrane and choriocapillaris may play a substantial role in pathogenesis of exudative AMD. However, little is known about the physiological function of these inhibitors in

development and function of choriocapillaris, and how their alterations contribute to the pathogenesis of AMD and CNV.

Thrombospondins

TSP1 or platelet TSP was the first member of the TSP family identified at high levels in the α -granules of platelets [24]. It is a homotrimeric multi-domain and multi-functional calcium-binding extracellular matrix protein, produced by many cell types including ChEC and RPE cells [25]. TSP1 and TSP2 are the only members of the TSP subfamily with antiangiogenic activity, but only TSP1 has been extensively studied [26]. TSP1 has six major motifs including N-terminal heparin binding domain, procollagen homology domain, type I repeats, type II repeats, type III repeats, and a C-terminal globular cell-binding domain. Domain organization of TSP1 and function of TSP1-derived peptides from these domains are shown in Figure 2. TSP2 also has a very similar multi-domain organization as TSP1, with the degree of homology with TSP1 increasing when moving from the N-terminus to C-terminus [27, 28].

The different biological functions of TSP1 is attributed to its interactions with various cell surface receptors that are differentially expressed in a cell type specific manner [29]. Its anti-angiogenic activity was the first identified biological function of TSP1 demonstrated in the late 1980s [30, 31]. TSP1 modulates proliferation, migration, differentiation, and apoptosis of various cell types including EC, pericytes, smooth muscle cells, fibroblasts and macrophages [31–33]. It also has a critical role in the regulation of various biological functions such as vascular homeostasis, immunity and wound healing [31, 34–36]. Although TSP2 exhibits some unique biological functions, it also shares some of the same biological functions as TSP1. However, the majority of shared functions, such wound healing, are not overlapping but specific to a TSP family member [37].

TSP1 and retinal vascularization

In eyes, TSP1 is present at high levels in the vitreous and aqueous humor from various species, and is a major component of Bruch's membrane [12, 13]. We have shown that TSP1 expression is required for adequate pruning and re-modeling of the developing retinal vasculature. TSP1 plays an active role in elimination of excess blood vessels during the late stages of retinal vascularization [38]. This effect is mitigated under ischemic conditions when VEGF levels are high. Thus, *Thbs1*-deficient mice fail to undergo appropriate vascular pruning and as a result exhibit increased vascular density during postnatal retinal vascular development [39, 40]. In addition, we showed that early and premature embryonic expression of TSP1 in the eye results in defective postnatal retinal vascular development and attenuation of retinal neovascularization during oxygen-induced ischemic retinopathy (OIR) [41].

Animal models have been extensively used in expanding our knowledge and understanding of the pathogenesis of exudative AMD and the development of new effective treatments [42]. Decreased TSP1 levels in the Bruch's membrane and choroidal vessels during AMD suggest a potential regulatory role for TSP1 in pathogenesis of CNV [12]. We recently investigated the impact of TSP1 expression in a mouse model of laser-induced CNV. We

showed that *Thbs1* $-/-$ mice exhibit significantly larger neovascular lesions compared with wild type (*Thbs1* $+/+$) mice. The average CNV area per eye was significantly increased (~ 8 -fold) in TSP1 null mice ($23,750 \pm 4,200 \mu\text{m}^2$, $n=15$ eyes) compared with WT mice ($3,235 \pm 910 \mu\text{m}^2$, $n=15$ eyes) [43]. The increased area of neovascularization was associated with increased recruitment of macrophages in *Thbs1* $-/-$ mice following laser rupture of Bruch's membrane. These results are consistent with the anti-angiogenic and anti-inflammatory activity of TSP1 [44–46] and support the hypothesis that aberrant modulation of TSP1 expression contributes to pathogenesis of exudative AMD [47].

Cell autonomous function of TSP1

Studies from our laboratory have demonstrated that TSP1 differentially affects the function of various ocular cell types which produce TSP1 including EC (Table 1). Retinal EC prepared from TSP1-deficient mice exhibit a pro-angiogenic phenotype through sustained activation of pro-angiogenic signaling pathways [31]. TSP1 regulates VEGF-A-mediated Akt signaling and capillary survival during retinal vascular development [48]. We have shown that *Thbs1* $-/-$ retinal EC are more proliferative and migratory [49]. In contrast, *Thbs1* $-/-$ choroidal EC are less proliferative and migratory [50]. We also recently showed significant changes in several aspects of RPE cell function associated with TSP1-deficiency, which may contribute to AMD pathogenesis (Table 1). *Thbs1* $-/-$ RPE cells exhibited increased proliferation and oxidative stress, and were less migratory. The increased proliferation rate of RPE cells in the absence of TSP1 suggest that TSP1 may negatively impact RPE cell proliferation, as in retinal EC, via CD47 and CD36 receptors, both of which are expressed in RPE cells. Increased macular densities of aging RPE cells is associated with higher apoptotic activity in the macula [51–53]. This increased death of macular RPE cells may be compensated by migration of peripheral RPE cells [52, 54]. We showed that TSP1 deficiency results in reduced migration of RPE cells. Thus, decreased level of TSP1 during AMD may decrease migration of RPE cells and promote RPE atrophy and progression of the disease.

The increased production of VEGF plays a substantial role in the development and progression of AMD and CNV. TSP1-deficiency resulted in significant changes in the level of VEGF produced by RPE cells further supporting a significant role for TSP1 changes in the development and progression of AMD. The roles of TSP1 in the regulation of angiogenic processes are complex and involve either direct or indirect effects on ocular vascular cells or their extracellular matrix composition. TSP1 conveys its anti-angiogenic effect via CD36, a scavenger receptor, and CD47 expressed on the EC [55–58]. However, the contribution of these interactions to RPE and choroidal EC function need further investigation.

Another biological feature exhibited in mice lacking TSP1 was increased infiltration of macrophages during early stage of laser-induced CNV, a sign of enhanced inflammation [43]. TSP1 modulates retinal vascular hemostasis and perfusion by regulation of nitric oxide (NO) signaling [31]. NO is a signaling molecule which plays a major role in vasodilatation and permeability [59]. Experimental findings indicated that increased NO production favors the formation of CNV leading to the development of AMD pathologies [60, 61]. Our laboratory previously reported that *Thbs1* $-/-$ choroidal EC exhibit higher level of

phosphorylated (active) eNOS and a significant increase in intracellular NO levels. In addition, *Thbs1* $-/-$ choroidal EC showed significantly higher levels of iNOS, a marker of inflammation, compared to the wild type cells. Elevated iNOS expression may result in significant induction of NO production and oxidative stress through production of peroxynitrate [50, 62–64]. These observations are consistent with the pro-inflammatory phenotype observed in *Thbs1* $-/-$ mice and enhanced laser-induced CNV [43].

TSP2 and retinal vascularization

The early studies examining TSP expression indicated that TSP2 is not expressed in mouse eyes [65]. However, we showed that TSP2 is not only expressed in retinal astrocytes but its level is increased in TSP1-deficient retinal astrocytes in culture [66]. We later showed that although TSP2 is undetectable in wild type retinal EC, wild type lung EC express significant amount of TSP2 [67]. In addition, *Cyp1b1*-deficient retinal EC expressed high levels of TSP2. This was attributed to increased oxidative stress in *Cyp1b1*-deficient retinal EC, and it was reversed by re-expression of *Cyp1b1* or incubation with the antioxidant N-acetylcysteine [68–70]. These studies suggested an important anti-angiogenic role for TSP2 under oxidative stress. We later showed that the retinal pericytes also produce TSP2, and in fact showed that pericytes are the major source of TSP2 production in retinal vasculature using a TSP2 reporter mouse [71]. The *Cyp1b1*-deficient retinal pericytes expressed higher TSP2 level and they were more proliferative and migratory [69].

Later we showed that TSP2 is also expressed in the mouse retina at postnatal day 5 (P5) and its level increased by nearly 3-fold and remained high up to P42. This was further confirmed by using wholemount staining and Western blot analysis of TSP2-GFP reporter mice. However, TSP2 protein levels (GFP-reporter) were undetectable at P42 [72]. This is consistent with limited expression of TSP2 protein observed in adult retina. We also showed that choroidal EC, unlike retinal EC, express a significant amount of TSP2. Furthermore, the expression of TSP2 was increased in *Thbs1*-deficient ChEC [50]. RPE cells also expressed a low amount of TSP2, which was increased in *Thbs1*-deficient RPE cells [73]. However, the cell autonomous impact of TSP2 expression on choroidal EC and RPE cell functions remain a subject of future investigation.

We have examined the impact of TSP2 expression on postnatal development of retinal vasculature, and retinal and choroidal neovascularization during OIR and laser-induced CNV, respectively. We showed that the primary retinal vasculature develops at a faster rate in *Thbs2* $-/-$ mice with a minimal impact on the rate of vascular cell proliferation and apoptosis. *Thbs2*-deficiency also minimally affected the degree of retinal and choroidal neovascularization [72]. However, TSP2 level was significantly up-regulated in P17 mice subjected to OIR. This could be attributed to increased oxidative stress at this stage and needs further verification. We also showed that although lack of TSP1 had no effect on expression of TSP2 level during normal development or OIR in the retina, the level of TSP1 was significantly lower in the absence of TSP2 [72]. Thus, lack of TSP2 expression in the retina does not cause a compensatory increase in TSP1 levels. These observations are consistent with different roles proposed for TSP1 and TSP2 in regulation of angiogenesis during normal development (TSP2) and reparative processes (TSP1).

The potential therapeutic use of TSP2 as an inhibitor of angiogenesis has not been extensively evaluated. Although the presence of similar anti-angiogenic domains and sequence homology suggest the presence of potential peptides from these regions that may have antiangiogenic and thus therapeutic potential, the availability and utility of such peptides awaits further investigation. Studies evaluating comparable c-terminal peptides from these molecules suggest significant differences among peptides from TSP1 and TSP2 [74]. These results are consistent with non-overlapping function of these members of TSP family and await further studies of TSP2 mimetic peptides.

PIGMENT EPITHELIUM DERIVED FACTOR (PEDF)

PEDF is a non-inhibitory serpin proteinase that was originally detected in the human fetal RPE cell [75, 76]. PEDF is a broadly expressed multifunctional protein with crucial roles in various physiological and pathophysiological mechanisms involved in angiogenesis, neuroprotection, fibrogenesis and inflammatory responses [77]. Neurotrophic or antiangiogenic activity of PEDF is determined by its phosphorylation state via casein kinase (CK2) and protein kinase A (PKA) [78]. Phosphorylation of PEDF by CK2 on Ser24 and Ser114 results in the antiangiogenic activity and a conformational change in PEDF that inhibits PEDF phosphorylation by PKA and eliminates its neurotrophic activity. PKA phosphorylates PEDF at Ser227 resulting in the neurotrophic activity of PEDF and reduced antiangiogenic activity [78]. Structural properties of PEDF and the function of PEDF-derived peptides from these domains are shown in Figure 3. PEDF exhibits a potent inhibitory activity toward angiogenic activity of VEGF and fibroblast growth factor (FGF). PEDF is an endogenous inhibitor of angiogenesis, is present at high levels in the eye, and is capable of blocking the activity of proangiogenic factors. PEDF is postulated to have a major role in neurovascular homeostasis and prevention of angiogenesis in healthy ocular tissue [79]. However, the detailed molecular and cellular mechanisms involved remain largely unknown.

PEDF and retinal vascularization

Altered production of PEDF plays a critical role in retinal differentiation and maintenance of retinal function as well as survival of retinal neurons via its neurotrophic function [80–82]. In the eye, PEDF modulates blood vessel growth by initiating a permissive environment for angiogenesis while oxygen concentration is low (such as retinopathies and/or tumors) and an inhibitory environment when oxygen concentrations are normal or high [83]. Previous studies in our laboratory investigated the role of PEDF in normal postnatal vascularization of retina and retinal neovascularization during OIR using PEDF deficient (*Serpinf1* $-/-$) mice. High oxygen exposure during OIR results in downregulation of proangiogenic factors and vessel obliteration, and halts further retinal vascular development [84]. When animals are returned to room air, the ischemic retina promote excessive production of angiogenic factors and subsequent abnormal new blood vessel growth. We showed that the primary retinal vasculature developed at a faster rate in *Serpinf1* $-/-$ mice. In addition, increased retinal microvessel density is reported in *Serpinf1* $-/-$ mice at 3 weeks of age [84]. PEDF deficiency was also associated with a more severe hyperoxia-mediated vessel obliteration during OIR with a significant effects on retinal neovascularization [84].

We also examined the impact of PEDF deficiency on CNV in a mouse model of laser induced CNV. Our results showed no significant differences in degree of CNV observed in *Serpinf1* $-/-$ mice compared with *Serpinf1* $+/+$ mice (Figure 4). Zhang and associates reported PEDF acts as a protective factor for retinal EC tight junctions. They showed that intravitreal injection of PEDF reduced retinal vascular permeability in rats with OIR and diabetes [85]. In addition, over-expression of PEDF resulted in dramatic suppression of retinal neovascularization during OIR [86]. Similarly, intravitreal injection of adeno-associated virus encoding PEDF significantly inhibited retinal neovascularization during OIR and choroidal neovascularization during laser induced CNV [87]. Thus, exogenously administered PEDF has a dramatic anti-angiogenic activity and may have therapeutic potential for inhibition of neovascularization.

PEDF also plays an important neuroprotective role, and promotes the survival of rod photoreceptor cells under degenerative conditions. In vivo studies have indicated that PEDF treatment can drastically delay the progression of photoreceptor degeneration in rodents with retinal degeneration mutations [81, 82, 88]. Changes in and loss of photoreceptors are also reported during AMD, specifically in patients with geographic atrophy [89, 90]. This may suggest a potential link between PEDF loss and photoreceptor degeneration during AMD development. However, we did not observe any sign of photoreceptor degeneration in PEDF deficient mice up to a year of age (Our unpublished data). The neuroprotective effect of PEDF is also reported by others [88, 91, 92]. Administration of exogenous PEDF decreases motor neuron death and protects survival of neurons from atrophy in neonatal mice exposed to sciatic nerve section [93]. However, we have observed a subtle, but not significant, increase in degenerative effect of PEDF deficiency on retinal ganglion cells (Figure 5). Thus, lack of PEDF minimally affected the degeneration of retinal ganglion cells in an optic nerve crush model. Therefore, the impact of endogenous PEDF deficiency may be compensated by other developmental changes minimizing its effect on retinal neurovascular development and function.

Cell autonomous function of PEDF

PEDF was first identified as a secreted protein in conditioned medium from RPE cells, which stimulated neuronal differentiation of Y79 retinoblastoma cell [94]. Soon after, PEDF was recognized as one of the major endogenous inhibitors of angiogenesis among other well defined antiangiogenic factors like TSP1, endostatin and angiostatin [83]. PEDF conveys its anti-angiogenic activity via PEDF receptors mainly by targeting EC. PEDF has a potent activity in inhibition of proliferation and migration of EC. PEDF as a trophic factor may also contribute to attenuation of proliferation by decreasing the number of cells entering S phase of the cell cycle and increasing the number of cells entering G0 [88, 95]. Dawson et al showed that PEDF inhibits EC migration toward different pro-angiogenic factors including VEGF, platelet-derived growth factor (PDGF), interleukin-8 (IL8), acidic fibroblast growth factor, and lysophosphatidic acid [83]. It is suggested that PEDF stimulates Fas ligand (FasL) expression and activates FAS/FASL transduction cascade leading to the EC death [96].

Studies from our laboratory demonstrated that PEDF differently affects the function of various cell types in the eye including EC and RPE cells (Table 2). PEDF also participates in the RPE cell differentiation and maturation [97]. We recently showed that PEDF deficiency greatly affected RPE cell proliferation, migration, adhesion, oxidative state, and phagocytic activity with minimal effect on their basal rate of apoptosis [73]. Altered migration of RPE cells in the absence of PEDF supports its significant contribution in RPE cell degeneration and the pathogenesis of AMD. Similar to RPE cells, choroidal and retinal EC lacking PEDF demonstrated a decreased migratory phenotype (Our unpublished data). This is consistent with the inability of *Serpinf1* $-/-$ choroidal and retinal EC to undergo capillary morphogenesis in Matrigel. Thus, expression of PEDF has a significant impact on choroidal and retinal EC phenotype.

The phagocytic function of RPE cells plays a critical role in elimination of toxic metabolic waste as well as RPE cell survival [10, 98, 99]. We reported that PEDF deficiency results in enhanced accumulation of phagocytized materials inside RPE cells. This was associated with impaired proteasome activity of *Serpinf1* $-/-$ RPE cells, including decreased caspase-like and trypsin-like activity [73]. Oxidative stress is identified as a crucial factor in the progression of AMD pathogenesis. Loss of RPE defensive mechanisms to cope with oxidative stress can advance retinal degeneration and/or ocular neovascularization [100, 101]. PEDF inhibits apoptosis induced by oxidative stress in retinal pericytes preventing their dysfunction [102]. We showed elevated oxidative stress levels in the *Serpinf1* $-/-$ RPE cells [73] and choroidal and retinal EC (Our unpublished data). Cao and associates showed that pretreatment of retinal cultures with PEDF inhibited mitochondrial signal transduction and induced apoptosis when challenged with H_2O_2 [82]. PEDF can also activate NF- κ B signaling pathway resulting in induction of anti-apoptotic and/or neurotrophic factors involved in cell survival [88]. Thus, it appears that PEDF can modulate various signal transduction pathways, perhaps in a tissue specific manner, through its interaction with different receptors on the cell surface.

PEDF ligand/receptor interactions are regulated by heparin and heparan sulfate which promote conformational changes allowing PEDF to bind to its receptor [103, 104]. Two major PEDF receptors have been identified. ATGL (PEDFR) is an 80 kDa protein and is found on motor neurons with a high affinity for the 44-mer PEDF peptide, which is involved in neurotrophic activity. Laminin receptor (LR) is a 60 kDa protein that binds PEDF and is mainly localized on EC. LR has a high affinity for the 34-mer PEDF peptide involved in inhibition of angiogenesis [105–107]. Regulation of PEDF activity by its specific receptors suggest the use of these receptors and downstream events as target for therapeutic activities. Retinal and choroidal EC, and RPE cells express both PEDF receptors, PEDFR and LR. However, the level of LR was (30- to 100-fold) higher than PEDFR in these cells (Our unpublished data). RPE cells expressed higher levels of PEDF receptors compared with retinal and choroidal EC. Retinal EC also expressed other PEDF receptors including PLXDC2 and PLXDC1 at significantly lower levels (300- and 15,000-fold) than LR (Our unpublished data). Development of antibodies or drugs targeting these receptors and their downstream effectors will provide new treatment modalities for diseases with a neovascular component including cancer and ocular vascular disease such as exudative AMD.

PEDF is also identified as a potent inhibitor of canonical Wnt signaling pathway [108]. The importance of Wnt signaling in various biological processes including inflammation, angiogenesis and fibrosis has been previously discussed [109, 110]. Activation of Wnt signaling plays an important role in oxidative stress, inflammation, and neovascularization associated with diabetic retinopathy and exudative AMD [100, 111]. The activation of Wnt signaling can be attenuated by induced expression of PEDF or administration of exogenous PEDF, as well as other antagonists of this pathway with significant impact on neovascularization. These observations further emphasize the important role of PEDF in pathogenesis of AMD and its potential utilization as target for treatment of exudative AMD.

TSP1 AS POTENTIAL THERAPEUTIC TARGET FOR EXUDATIVE AMD

We were first to report the presence of TSP1 and its antiangiogenic fragment in vitreous and aqueous humor samples of normal human, rat, mouse and bovine eyes [13]. In addition, we showed a dramatic decrease in its level in samples from diabetic rats [13]. Since TSP1 is an endogenous inhibitor of angiogenesis with potential significant clinical impact in pathogenesis of many diseases with a neovascular component, many studies have explored its therapeutic applications providing us with a better understanding of the detailed mechanisms of TSP1 action. The results of these studies facilitated the design of therapeutic strategies to optimize TSP1 function and efficacy. Up-regulation of endogenous TSPs, synthetic TSP1 peptides, recombinant proteins derived from the angiogenic fragment of TSP1, or both, are used to evaluate TSP1 therapeutic effects [28, 40, 45, 112]. Thus, it is important to know the specific function of the TSP1 derived peptides to gain insight into the biological, physiological and pathological function of TSP1. TSP1 expression is required for adequate pruning and re-modeling of the retinal vasculature during development and maintenance of the quiescent state of vasculature [31]. Therefore, alterations in TSP1 levels during AMD may significantly affect ocular vascular hemostasis.

The synthetic peptides from TSP1 have been extensively used in various tumor models in vitro and in vivo [40, 113–115]. Peptides derived from the properdin- type1 and procollagen homology region of TSP1 attenuate CNV in mice [113]. Several investigators have suggested that TSP1 may also directly contribute in suppression of cancer cell proliferation [33, 40]. ABT-510 was developed to improve the pharmacodynamic and pharmacokinetic activity of the TSP1 type 1 repeat peptide [113]. ABT-510 is a nonapeptide analogue of an antiangiogenic sequence with a single D-amino-acid substitution that initiates 1,000-fold greater antiangiogenic activity. TSP1 is reported as a protective factor in mice with inflammatory bowel disease (IBD) treated with ABT- 510 [116]. ABT-510 impacts angiogenesis mechanisms in a negative manner through competition with TSP1 for binding to EC, induction of Fas ligand expression in EC, and inhibition of VEGF activity [117].

Synthetic manipulations have improved the serum half- life of ABT-510 (NAC SarGlyValDalloIleThrNvalleArgProNHE) including substitution of the first Ile of GVITRIR with Dlle or DalloIle, and the first Arg with norvaline, capping of the terminal amino- and carboxyl-residues [113]. Treatment with ABT-510 increases the apoptotic rate of bovine capillary EC, and human umbilical artery EC to inhibit capillary morphogenesis induced by VEGF, as well as inhibition of CNV induced by basic fibroblast growth factor (bFGF) [113,

118]. Anti-angiogenic mimetic peptides from TSP1 have been extensively used in several preclinical tumor models [40, 113–115, 119]. ABT- 510 is a protective factor in mice with Lewis lung carcinoma, bladder and prostate cancer [113, 116, 118]. ABT-898 is a more recent generation of TSP1 mimic peptide, which successfully decreased tumor size in mice with uveal melanoma [40]. Thus, TSP1 mimetic peptides are efficacious in attenuating angiogenesis and tumor growth.

Studies by our group and others support the strong mimicry activity of TSP1 peptides via normalizing angiogenesis with the TSP1 deficiency due to loss or downregulation. For example, CNV area is approximately 8- fold larger in *Thsp1* $-/-$ mice compared with wild type mice [43]. This increase was abolished by intravitreal administration of TSP1 mimetic peptide ABT-898. ABT-898 is a TSP1 octapeptide, which has slower clearance, 10-fold greater potency and no enzymatic cleavage [120]. Furthermore, systemic administration of ABT-898 effectively attenuated tumor growth in a mouse model of uveal melanoma. The average tumor volume in TSP1 peptide-treated mice decreased by approximately 10-fold compared with a control group [40].

Phase I trial results showed ABT-510 is well tolerated with biologically relevant plasma concentrations (>100 ng/mL lasting at least 3 h/d) and dose proportional, time independent pharmacokinetics [121–123]. ABT-510 has been evaluated in phase II clinical trials for the treatment of renal cell carcinoma, soft tissue sarcoma, lymphoma, non-small cell lung cancer, and head and neck cancer [124]. The therapeutic benefits of ABT-510 was demonstrated in several malignancies [33]. However, randomized ABT-510 treatment of patients with advanced renal cell carcinoma indicated limited clinical activity [117].

Combining ABT-510 with other standard treatments is feasible. ABT-510 combined with valporic acid (VPA, histone deacetylase inhibitor) effectively inhibited the growth of small neuroblastoma xenografts through significant reduction of microvascular density, suggesting that this combined regimen may be an effective antiangiogenic therapy for children with high-risk neuroblastoma [33]. Combination of ABT-510 with gemcitabine-cisplatin, 5-fluorouracil and leucovorin (5-FU/LV) did not appear to increase toxicity and pharmacokinetic interactions [125]. Our studies indicated that TSP1 mimetic peptides (ABT-510 and ABT-898) attenuate CNV in pre-clinical models. TSP1 blocks angiogenesis through specific apoptosis mechanisms that do not duplicate that of VEGF antagonists. Thus, TSP1 mimetic peptides have the potential to act synergistically and to enhance activity of VEGF antagonists to mitigate CNV. Collectively, based on the promising pre-clinical data provided above, TSP1 peptides are a promising treatment to prevent or arrest CNV progression in patients with exudative AMD and await human trials.

PEDF AS POTENTIAL THERAPEUTIC TARGET FOR EXUDATIVE AMD

Endogenous management of blood vessel growth is regulated through proangiogenic factors including VEGF family and anti-angiogenesis factors including TSP1 and PEDF. PEDF is recognized as a major endogenous inhibitor of neovascularization. PEDF, a 50 kDa glycoprotein, is a member of serpin superfamily with no serine protease inhibitor activity [76]. PEDF is a multifunctional protein that has broad spectrum of activities including

neurotrophic, neuroprotective, anti-inflammatory, anti-tumorigenic, anti-angiogenic and anti-vasopermeability [79, 83, 126]. The inhibitory impact of PEDF on angiogenesis was demonstrated by inhibition of VEGF and FGF activity, only on newly forming blood vessels without affecting pre-existing blood vessels. However, the molecular mechanisms that PEDF utilizes to convey its anti-angiogenesis activity are not very well understood. A recent study reported a direct interaction between PEDF and VEGF receptors which suggest a potential mechanism for the inhibition of angiogenesis [127]. Selective induction of apoptosis in EC of newly forming blood vessels, and its broad distribution and action in different cell types, make PEDF a suitable candidate for anti-angiogenesis therapy. Published PEDF patents have recommended the use of full length PEDF, derived peptides or PEDF gene delivery as anti-cancer drugs [128, 129]. Therapeutic effects of PEDF in prevention or treatment of melanoma and osteosarcoma cancers are shown in some patent applications [130, 131].

Introducing exogenous PEDF to a neovascularized area results in the inhibition of both EC migration and proliferation through induction of apoptosis of recruited EC [132–134]. Re-expression of PEDF in the human esophageal squamous cell carcinoma that normally do not secrete the endogenous PEDF, significantly inhibited their migration and proliferation and halted their growth by suppressing neovascularization [135]. Similarly, constitutive over-expression of PEDF decreased the size of ocular tumors metastasis associated with reduced microvascular density in pre-clinical models [136]. In addition, PEDF over-expression decreased migration and capillary morphogenesis of melanoma cells in vitro [136]. Thus, exogenous administration of PEDF is effective in attenuating angiogenesis and tumor growth.

PEDF is also efficacious in the attenuation of ocular neovascularization. Dawson et al showed that purified, as well as recombinant PEDF, attenuated corneal neovascularization in rats [83]. In another study using a mouse model of ischemia-induced retinopathy, low doses of recombinant PEDF blocked retinal neovascularization without affecting the pre-existing blood vessels and retinal morphology [137]. Intravitreal injection of AdPEDF.11, an adenovirus encoding PEDF, showed significant inhibition of CNV which corresponded with induced apoptosis of EC within the neovascularized area [138]. AdPEDF.11D was tested in an open-label, dose escalation phase I trial to identify the maximum tolerated dose (MTD) and assess safety and potential efficacy in patients with advanced exudative AMD [139]. The result of this study indicated that intravitreal injection of AdPEDF.11D is a feasible approach for the treatment of ocular diseases, since no severe adverse effects in the patients were reported. However, further investigation to evaluate the efficacy of AdPEDF.11 in patients with exudative AMD is needed [140]

PEDF mimetic peptides derived from the N-terminal anti-angiogenic epitope showed potent anti-angiogenic activity and PEDF mimicry including apoptosis and major signaling pathways in models of tumor angiogenesis, wound healing and CNV in mice. PEDF peptides have potent anti-angiogenic effects in orthotopics models of prostate and renal cancers [141, 142]. PEDF and PEDF derived peptide 44mer have inhibitory effects against hypoxia induced apoptosis in cardiomyocytes [143]. Studies reported mimicry activity of PEDF 34 and PEDF 18 peptides could inhibit angiogenesis through cellular and molecular events characteristic of anti-angiogenesis of PEDF [141, 142, 144]. In our preliminary

studies, we have observed therapeutic efficacy of a PEDF18 derived peptide in a mouse CNV model (Our unpublished data). Thus, for exudative AMD, faced with lack of suitable options for lasting control over a progressive condition, along with adverse effects of current treatments on ocular function and systemic side effects, the use of endogenous biological agents provide great opportunity for new treatment modality [145–147].

Endostatin

Endostatin is a 25 kDa protein derived from C-terminal region of collagen XVIII, a heparin sulfate proteoglycan (HSPG) core protein [148, 149]. It is a potent endogenous inhibitor of angiogenesis, which presents dual receptor antagonism resulting in anti-angiogenesis and pro- autophagy activity [149]. Endostatin was originally characterized by its inhibitory effect on EC and suppressive tumor-induced angiogenesis [21]. Endostatin conveys its biological activities through multiple interacting receptors. It can bind $\alpha 5\beta 1$, and $\alpha v\beta 3/\alpha v\beta 5$ integrins and antagonize their activity on EC. Furthermore, direct interaction of endostatin with $\alpha 5\beta 1$ integrin leads to induced autophagy in EC that enhances its anti-angiogenic activity [150]. Endostatin binds to cell surface HSPG glypican, VEGFR1 and VEGFR2, and fibronectin receptor $\alpha 5\beta 1$ integrin [151–154]. Endostatin inhibits VEGF induced phosphorylation by binding directly to VEGFR2 [152]. The anti-angiogenic activity of endostatin was initially identified by its inhibitory impact on blood vessel formation in vivo and further confirmed by systemic administration of endostatin in tumor-bearing mice [21]. Endostatin utilizes broad mechanisms of action to exert its angiostatic activity on EC. The proposed mechanisms include inhibition of matrix metalloproteinase (MMP) activity, a facilitator of migration and invasion of EC and actin disassembly, inhibition of the FAK/Ras/p38-MAPK/ERK signaling cascade via binding to $\alpha 5\beta 1$ -integrin, suppression of HIF-1 α /VEGFA, and Wnt signaling [155–161].

Altered endostatin levels is associated with different diseases including cancers, vascular and neurological diseases [21, 148, 162, 163]. Decreased level of endostatin occurs in Bruch's membrane and/or choriocapillaris during AMD and retinopathies [5]. Involvement of endostatin as an endogenous inhibitor of angiogenesis in the pathology of various diseases suggests its potential clinical applications through development of novel therapeutics. Currently, clinical endostatin use in the US is in a Phase I trial in patients with advanced exudative AMD [164]. Several research groups suggest that the clinical applications of endostatin in a form of short peptides alone or in combination with other therapy regimens will be a great therapeutic option for treatment of various diseases with a neovascular component [148].

Angiostatin

Angiostatin is an internal fragment of plasminogen and includes four kringle domains (k1-4) [165]. Kringle structure exists in various proteins and was originally described by a triple loop structure linked by three pairs of disulfide bonds [166]. These kringle domains show anti-angiogenic effect only when cleaved indicating proteolysis plays an important role in the angiogenesis balance by down regulation of angiogenesis [165]. Angiostatin inhibits EC proliferation and EC capillary cell growth [167]. The suppressive impact of angiostatin on the cell growth is mostly restricted to the EC lineage [168]. Angiostatin has an inhibitory

impact on metastatic tumor growth mainly through blocking of angiogenesis [167]. Studies have reported that different proteases produced by tumor cells can cleave plasminogen to release angiostatin [165]. Several mechanisms of action are proposed as potential pathways for anti-angiogenesis activity of angiostatin including suppression of EC migration and proliferation via binding to ATPase and/or prevention of the G2/M transition, induced apoptosis of EC, and down regulation of VEGF expression [152, 167, 169–171]. Angiostatin has anti-tumor activity in different animal models, and has been clinically evaluated in phase I and II clinical trials in patients with progressive metastatic cancer and non-small-cell lung cancer [172]. Decreased level of angiostatin in Bruch's membrane and/or the choriocapillaris complex during AMD has been reported [5]. Currently, angiostatin is tested in the open-label, dose escalation phase I trial to identify the maximum tolerated dose and assess safety and potential efficacy in patients with advanced exudative AMD [164].

CONCLUSIONS AND FUTURE DIRECTIONS

Age-related macular degeneration is a major cause of visual impairment in the elderly population worldwide. Despite the high prevalence of AMD, its etiology remains largely unknown. The increased production of VEGF is identified as an essential factor in development and progression of AMD, and CNV. Intravitreal injection of VEGF antagonists is effective in delaying CNV and pathological progression of the disease. However, safety concerns are emerging regarding long term blockade of VEGF, and loss of ocular integrity due to multiple intravitreal injections and systemic complications. Other major complications associated with chronic use of anti-VEGF include intraocular inflammation, rhegmatogenous retinal detachment, intra ocular pressure elevation, ocular hemorrhage and vascular degeneration [173–179]. In addition, there are several reports indicating RPE tears associated with intravitreal anti-VEGF delivery in patients with exudative AMD [180–182]. Thus, safe and effective alternative treatments are needed. PEDF and TSP1 are major endogenous inhibitors of ocular angiogenesis, which are deficient in vascular retinopathies including human AMD. Small peptides derived from their active fragments are potentially anti-angiogenic in preclinical models of ocular diseases and tumors, and restore a normal PEDF and TSP1 status. Our preliminary studies indicate that these peptides attenuate CNV in pre-clinical models of exudative AMD [40, 41].

PEDF and TSP1 block angiogenesis through specific apoptosis mechanisms, which are different from the mechanisms of VEGF antagonists. Thus, PEDF and TSP1 mimetic peptides may act synergistically and enhance the effects of VEGF antagonists. Understanding the role of PEDF and TSP1, and their signaling pathways in ocular vascular homeostasis is instrumental in the development of new modalities for the treatment of exudative AMD. In addition, evaluation of the potential therapeutic action of TSP1 and PEDF peptides for their mimicry activities of these natural endogenous inhibitors will provide further justification for treatment of CNV. Further exploration into combination therapy of VEGF antagonists and PEDF and/or TSP1 peptides will allow the assessment of their synergistic effect on inhibition of CNV. These approaches will provide an opportunity to identify potential new therapeutic targets for AMD treatment as well as identifying a single, most promising clinically candidate for human clinical trials.

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References

1. Lally DR, Gerstenblith AT, Regillo CD. Preferred therapies for neovascular age-related macular degeneration. *Curr Opin Ophthalmol*. 2012; 23(3):182–8. [PubMed: 22450218]
2. Ambati J, et al. Age-Related Macular Degeneration: Etiology, Pathogenesis, and Therapeutic Strategies. *Survey of Ophthalmology*. 2003; 48(3):257–293. [PubMed: 12745003]
3. Hua JGN, Schulze B, Michaelis U, Bohnenkamp H, Guenzi E, Hansen L, Martin G, Hansjürgen T. In vivo imaging of choroidal angiogenesis using fluorescence-labeled cationic liposomes. *Mol Vis*. 2012; 18:1045–1054. [PubMed: 22605917]
4. Jung JJ, et al. The Incidence of Neovascular Subtypes in Newly Diagnosed Neovascular Age-Related Macular Degeneration. *American Journal of Ophthalmology*. 2014; 158(4):769–779.e2. [PubMed: 25034111]
5. Bhutto I, Luty G. Understanding age-related macular degeneration (AMD): relationships between the photoreceptor/retinal pigment epithelium/Bruch's membrane/choriocapillaris complex. *Mol Aspects Med*. 2012; 33(4):295–317. [PubMed: 22542780]
6. Green WR. Histopathology of age-related macular degeneration. *Mol Vis*. 1999; 5:27. [PubMed: 10562651]
7. Green WR, Enger C. Age-related macular degeneration histopathologic studies. The 1992 Lorenz E. Zimmerman Lecture. *Ophthalmology*. 1993; 100(10):1519–35. [PubMed: 7692366]
8. Campochiaro PS, Hafiz M, Heier G, Lit J, Zimmer-Galler E, Channa I, Nguyen R, Syed QD, Do B, Lu D, Monk L, Cooke, Kengatharan J, Hsu KH. Topical Mecamylamine for Diabetic Macular Edema. *Am J Ophthalmol*. 2010; 149(5):839–51. [PubMed: 20189159]
9. Duh EJ, et al. Vitreous levels of pigment epithelium-derived factor and vascular endothelial growth factor: Implications for ocular angiogenesis. *American Journal of Ophthalmology*. 2004; 137(4):668–674. [PubMed: 15059706]
10. Sparrow HD JR, Hamel CP. The retinal pigment epithelium in health and disease. *Curr Mol Med*. 2010; 10:802–823. [PubMed: 21091424]
11. Wang S, et al. Modulation of thrombospondin 1 and pigment epithelium-derived factor levels in vitreous fluid of patients with diabetes. *Arch Ophthalmol*. 2009; 127(4):507–13. [PubMed: 19365032]
12. Uno KBIA, McLeod DS, Merges C, Luty GA. Impaired expression of thrombospondin-1 in eyes with age related macular degeneration. *Br J Ophthalmol*. 2006; 90:48–54. [PubMed: 16361667]
13. Sheibani N, et al. Thrombospondin-1, a natural inhibitor of angiogenesis, is present in vitreous and aqueous humor and is modulated by hyperglycemia. *Biochem Biophys Res Commun*. 2000; 267(1):257–61. [PubMed: 10623607]
14. Karakousis PC, et al. Localization of pigment epithelium derived factor (PEDF) in developing and adult human ocular tissues. *Molecular Vision*. 2001; 7(22):154–163. [PubMed: 11438800]
15. Yamagishi SI, et al. Pigment epithelium-derived factor inhibits leptin-induced angiogenesis by suppressing vascular endothelial growth factor gene expression through anti-oxidative properties. *Microvascular Research*. 2003; 65(3):186–190. [PubMed: 12711260]
16. Ma XY, et al. Microphthalmia-associated transcription factor acts through PEDF to regulate RPE cell migration. *Experimental Cell Research*. 2012; 318(3):251–261. [PubMed: 22115973]

17. Machali ska AS, KMPK, Dziedzicko V, Karczewicz D. PEDF and VEGF plasma level alterations in patients with dry form of age-related degeneration—a possible link to the development of the disease. *Klin Oczna*. 2012; 114:115–120. [PubMed: 23346798]
18. Folkman J, Klagsbrun M. Angiogenic factors. *Science*. 1987; 235(4787):442–447. [PubMed: 2432664]
19. Gao G, et al. Unbalanced expression of VEGF and PEDF in ischemia-induced retinal neovascularization. *FEBS Lett*. 2001; 489(2–3):270–6. [PubMed: 11165263]
20. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature*. 2000; 407(6801):249–257. [PubMed: 11001068]
21. O'Reilly MS, et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell*. 1997; 88(2):277–85. [PubMed: 9008168]
22. Carron JA, et al. Cultured human retinal pigment epithelial cells differentially express thrombospondin-1, -2, -3, and -4. *Int J Biochem Cell Biol*. 2000; 32(11–12):1137–42. [PubMed: 11137453]
23. Miyajima-Uchida H, et al. Production and accumulation of thrombospondin-1 in human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci*. 2000; 41(2):561–7. [PubMed: 10670489]
24. Baenziger NL, Brodie GN, Majerus PW. A thrombin-sensitive protein of human platelet membranes. *Proc Natl Acad Sci U S A*. 1971; 68(1):240–3. [PubMed: 5276296]
25. Adams JC. Thrombospondins: multifunctional regulators of cell interactions. *Annu Rev Cell Dev Biol*. 2001; 17:25–51. [PubMed: 11687483]
26. Vailhe B, Feige JJ. Thrombospondins as anti-angiogenic therapeutic agents. *Curr Pharm Des*. 2003; 9(7):583–8. [PubMed: 12570805]
27. Bornstein P. Thrombospondins: structure and regulation of expression. *The FASEB Journal*. 1992; 6(14):3290–3299. [PubMed: 1426766]
28. Armstrong LC, Bornstein P. Thrombospondins 1 and 2 function as inhibitors of angiogenesis. *Matrix Biol*. 2003; 22(1):63–71. [PubMed: 12714043]
29. Tolsma SS, et al. Peptides derived from two separate domains of the matrix protein thrombospondin-1 have anti-angiogenic activity. *J Cell Biol*. 1993; 122(2):497–511. [PubMed: 7686555]
30. Sheibani N, Frazier WA. Thrombospondin-1, PECAM-1, and regulation of angiogenesis. *Histol Histopathol*. 1999; 14(1):285–94. [PubMed: 9987673]
31. Sheibani, N., et al. *Signal Transduction in the Retina*. CRC Press; 2007. Thrombospondin-1 Signal Transduction and Retinal Vascular Homeostasis; p. 329–345.
32. Miyata Y, Sakai H. Thrombospondin-1 in urological cancer: pathological role, clinical significance, and therapeutic prospects. *Int J Mol Sci*. 2013; 14(6):12249–72. [PubMed: 23749112]
33. Yang Q, et al. Thrombospondin-1 peptide ABT-510 combined with valproic acid is an effective antiangiogenesis strategy in neuroblastoma. *Cancer Res*. 2007; 67(4):1716–24. [PubMed: 17308113]
34. Mochizuki M, Sugita S, Kamoi K. Immunological homeostasis of the eye. *Prog Retin Eye Res*. 2013; 33:10–27. [PubMed: 23108335]
35. Zamiri P, et al. Thrombospondin plays a vital role in the immune privilege of the eye. *Invest Ophthalmol Vis Sci*. 2005; 46(3):908–19. [PubMed: 15728547]
36. Mochizuki M. Regional immunity of the eye. *Acta Ophthalmol*. 2010; 88(3):292–9. [PubMed: 19900207]
37. Agah A, et al. The lack of thrombospondin-1 (TSP1) dictates the course of wound healing in double-TSP1/TSP2-null mice. *Am J Pathol*. 2002; 161(3):831–9. [PubMed: 12213711]
38. Kisselev, OG., et al. *Signal Transduction in the Retina*. CRC Press; 2007. Thrombospondin-1 Signal Transduction and Retinal Vascular Homeostasis; p. 329–345.
39. Wang S, et al. Thrombospondin-1-deficient mice exhibit increased vascular density during retinal vascular development and are less sensitive to hyperoxia-mediated vessel obliteration. *Dev Dyn*. 2003; 228(4):630–42. [PubMed: 14648840]

40. Wang S, et al. Suppression of thrombospondin-1 expression during uveal melanoma progression and its potential therapeutic utility. *Archives of ophthalmology*. 2012; 130(3):336–41. [PubMed: 22411663]
41. Wu Z, et al. Attenuation of retinal vascular development and neovascularization in transgenic mice over-expressing thrombospondin-1 in the lens. *Dev Dyn*. 2006; 235(7):1908–20. [PubMed: 16691615]
42. Evans J, Wormald R. Is the incidence of registrable age-related macular degeneration increasing? *The British Journal of Ophthalmology*. 1996; 80(1):9–14. [PubMed: 8664242]
43. Wang SJ, Sorenson CM, Sheibani N. Lack of Thrombospondin 1 and Exacerbation of Choroidal Neovascularization. *Archives of Ophthalmology*. 2012; 130(5):615–620. [PubMed: 22232368]
44. Lawler J. Thrombospondin-1 as an endogenous inhibitor of angiogenesis and tumor growth. *Journal of cellular and molecular medicine*. 2002; 6(1):1–12. [PubMed: 12003665]
45. Zhang X, Lawler J. Thrombospondin-based antiangiogenic therapy. *Microvasc Res*. 2007; 74(2–3): 90–9. [PubMed: 17559888]
46. Lopez-Dee Z, Pidcock K, Gutierrez LS. Thrombospondin-1: multiple paths to inflammation. *Mediators of inflammation*. 2011; 2011
47. Bhutto IA, et al. Reduction of endogenous angiogenesis inhibitors in Bruch's membrane of the submacular region in eyes with age-related macular degeneration. *Archives of Ophthalmology*. 2008; 126(5):670–678. [PubMed: 18474778]
48. Sun J, et al. Thrombospondin-1 modulates VEGF-A-mediated Akt signaling and capillary survival in the developing retina. *Am J Physiol Heart Circ Physiol*. 2009; 296(5):H1344–51. [PubMed: 19304944]
49. Wang Y, Wang S, Sheibani N. Enhanced proangiogenic signaling in thrombospondin-1-deficient retinal endothelial cells. *Microvasc Res*. 2006; 71(3):143–51. [PubMed: 16624339]
50. Fei P, et al. Expression of thrombospondin-1 modulates the angioinflammatory phenotype of choroidal endothelial cells. *PLoS One*. 2014; 9(12):e116423. [PubMed: 25548916]
51. Dorey CK, et al. Cell loss in the aging retina. Relationship to lipofuscin accumulation and macular degeneration. *Invest Ophthalmol Vis Sci*. 1989; 30(8):1691–9. [PubMed: 2759786]
52. Harman AM, et al. Development and aging of cell topography in the human retinal pigment epithelium. *Invest Ophthalmol Vis Sci*. 1997; 38(10):2016–26. [PubMed: 9331265]
53. Del Priore LV, Kuo YH, Tezel TH. Age-related changes in human RPE cell density and apoptosis proportion in situ. *Invest Ophthalmol Vis Sci*. 2002; 43(10):3312–8. [PubMed: 12356840]
54. Pan CK, et al. Embryonic stem cells as a treatment for macular degeneration. *Expert Opinion on Biological Therapy*. 2013; 13(8):1125–1133. [PubMed: 23705996]
55. Kaur S, et al. Thrombospondin-1 inhibits VEGF receptor-2 signaling by disrupting its association with CD47. *Journal of Biological Chemistry*. 2010; 285(50):38923–38932. [PubMed: 20923780]
56. Brown EJ, Frazier WA. Integrin-associated protein (CD47) and its ligands. *Trends in cell biology*. 2001; 11(3):130–135. [PubMed: 11306274]
57. Febbraio M, Hajjar DP, Silverstein RL. CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. *The Journal of clinical investigation*. 2001; 108(6):785–791. [PubMed: 11560944]
58. Clezardin P, et al. Expression of thrombospondin (TSP1) and its receptors (CD36 and CD51) in normal, hyperplastic, and neoplastic human breast. *Cancer research*. 1993; 53(6):1421–1430. [PubMed: 7680285]
59. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev*. 1991; 43(2):109–42. [PubMed: 1852778]
60. Ando A, et al. Nitric oxide is proangiogenic in the retina and choroid. *J Cell Physiol*. 2002; 191(1): 116–24. [PubMed: 11920687]
61. Ando A, et al. Blockade of nitric-oxide synthase reduces choroidal neovascularization. *Mol Pharmacol*. 2002; 62(3):539–44. [PubMed: 12181430]
62. Kibbe M, Billiar T, Tzeng E. Inducible nitric oxide synthase and vascular injury. *Cardiovasc Res*. 1999; 43(3):650–7. [PubMed: 10690336]

63. Leal EC, et al. Inducible nitric oxide synthase isoform is a key mediator of leukostasis and blood-retinal barrier breakdown in diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2007; 48(11):5257–65. [PubMed: 17962481]
64. Nagareddy PR, et al. Increased expression of iNOS is associated with endothelial dysfunction and impaired pressor responsiveness in streptozotocin-induced diabetes. *Am J Physiol Heart Circ Physiol*. 2005; 289(5):H2144–52. [PubMed: 16006542]
65. Suzuma K, et al. Expression of thrombospondin-1 in ischemia-induced retinal neovascularization. *The American journal of pathology*. 1999; 154(2):343–354. [PubMed: 10027393]
66. Scheef E, et al. Isolation and characterization of murine retinal astrocytes. *Mol Vis*. 2005; 11:613–624. [PubMed: 16148882]
67. Shin ES, Sorenson CM, Sheibani N. PEDF expression regulates the proangiogenic and proinflammatory phenotype of the lung endothelium. *Am J Physiol Lung Cell Mol Physiol*. 2014; 306(7):L620–34. [PubMed: 24318110]
68. Tang Y, et al. CYP1B1 and endothelial nitric oxide synthase combine to sustain proangiogenic functions of endothelial cells under hyperoxic stress. *American Journal of Physiology-Cell Physiology*. 2010; 298(3):C665–C678. [PubMed: 20032512]
69. Palenski TL, et al. Cyp1B1 expression promotes angiogenesis by suppressing NF- κ B activity. *American Journal of Physiology-Cell Physiology*. 2013; 305(11):C1170–C1184. [PubMed: 24088896]
70. Tang Y, et al. CYP1B1 expression promotes the proangiogenic phenotype of endothelium through decreased intracellular oxidative stress and thrombospondin-2 expression. *Blood*. 2009; 113(3):744–754. [PubMed: 19005183]
71. Scheef EA, Sorenson CM, Sheibani N. Attenuation of proliferation and migration of retinal pericytes in the absence of thrombospondin-1. *American Journal of Physiology-Cell Physiology*. 2009; 296(4):C724–C734. [PubMed: 19193867]
72. Fei P, et al. Thrombospondin-2 Expression During Retinal Vascular Development and Neovascularization. *Journal of Ocular Pharmacology and Therapeutics*. 2015; 31(7):429–444. [PubMed: 25950258]
73. Farnoodian M, et al. Expression of pigment epithelium-derived factor and thrombospondin-1 regulate proliferation and migration of retinal pigment epithelial cells. *Physiological reports*. 2015; 3(1):e12266. [PubMed: 25602019]
74. Isenberg JS, et al. Differential interactions of thrombospondin-1, -2, and -4 with CD47 and effects on cGMP signaling and ischemic injury responses. *J Biol Chem*. 2009; 284(2):1116–25. [PubMed: 19004835]
75. Becerra SP, et al. Pigment Epithelium-derived Factor Behaves Like a Noninhibitory Serpin NEUROTROPHIC ACTIVITY DOES NOT REQUIRE THE SERPIN REACTIVE LOOP. *Journal of Biological Chemistry*. 1995; 270(43):25992–25999. [PubMed: 7592790]
76. Becerra SP. Focus on molecules: pigment epithelium-derived factor (PEDF). *Experimental eye research*. 2006; 82(5):739–740. [PubMed: 16364294]
77. He X, et al. PEDF and its roles in physiological and pathological conditions: implication in diabetic and hypoxia-induced angiogenic diseases. *Clinical Science*. 2015; 128(11):805–823. [PubMed: 25881671]
78. Maik-Rachline G, Seger R. Variable phosphorylation states of pigment-epithelium-derived factor differentially regulate its function. *Blood*. 2006; 107(7):2745–2752. [PubMed: 16322471]
79. Bouck N. PEDF: anti-angiogenic guardian of ocular function. *Trends in molecular medicine*. 2002; 8(7):330–334. [PubMed: 12114112]
80. Volpert KN, et al. PEDF and GDNF are key regulators of photoreceptor development and retinal neurogenesis in reaggregates from chick embryonic retina. *Journal of ocular biology, diseases, and informatics*. 2009; 2(1):1–11.
81. Cayouette M, et al. Pigment epithelium-derived factor delays the death of photoreceptors in mouse models of inherited retinal degenerations. *Neurobiology of disease*. 1999; 6(6):523–532. [PubMed: 10600408]

82. Cao W, et al. Pigment epithelium-derived factor protects cultured retinal neurons against hydrogen peroxide-induced cell death. *Journal of neuroscience research*. 1999; 57(6):789–800. [PubMed: 10467250]
83. Dawson D, et al. Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science*. 1999; 285(5425):245–248. [PubMed: 10398599]
84. Huang Q, et al. PEDF-deficient mice exhibit an enhanced rate of retinal vascular expansion and are more sensitive to hyperoxia-mediated vessel obliteration. *Experimental eye research*. 2008; 87(3): 226–241. [PubMed: 18602915]
85. Zhang SX, et al. Pigment epithelium-derived factor (PEDF) is an endogenous antiinflammatory factor. *The FASEB journal*. 2006; 20(2):323–325. [PubMed: 16368716]
86. Park K, et al. Overexpression of Pigment Epithelium-Derived Factor Inhibits Retinal Inflammation and Neovascularization. *The American journal of pathology*. 2011; 178(2):688–698. [PubMed: 21281801]
87. Mori K, et al. Pigment epithelium-derived factor inhibits retinal and choroidal neovascularization. *Journal of cellular physiology*. 2001; 188(2):253–263. [PubMed: 11424092]
88. Barnstable CJ, Tombran-Tink J. Neuroprotective and antiangiogenic actions of PEDF in the eye: molecular targets and therapeutic potential. *Progress in retinal and eye research*. 2004; 23(5):561–577. [PubMed: 15302351]
89. Curcio CA. Photoreceptor topography in ageing and age-related maculopathy. *Eye (Lond)*. 2001; 15(Pt 3):376–83. [PubMed: 11450761]
90. Curcio CA, et al. Human photoreceptor topography. *Journal of Comparative Neurology*. 1990; 292(4):497–523. [PubMed: 2324310]
91. Ogata N, et al. Pigment epithelium derived factor as a neuroprotective agent against ischemic retinal injury. *Current eye research*. 2001; 22(4):245–252. [PubMed: 11462162]
92. Tombran-Tink J, Barnstable CJ. PEDF: a multifaceted neurotrophic factor. *Nature Reviews Neuroscience*. 2003; 4(8):628–636. [PubMed: 12894238]
93. Houenou LJ, et al. Pigment epithelium-derived factor promotes the survival and differentiation of developing spinal motor neurons. *Journal of Comparative Neurology*. 1999; 412(3):506–514. [PubMed: 10441236]
94. Tombran-Tink J, Johnson LV. Neuronal differentiation of retinoblastoma cells induced by medium conditioned by human RPE cells. *Investigative ophthalmology & visual science*. 1989; 30(8): 1700–1707. [PubMed: 2668219]
95. Hjelmeland LM, et al. Senescence of the retinal pigment epithelium. *Mol Vis*. 1999; 5(1):33–37. [PubMed: 10562657]
96. Volpert OV, et al. Inducer-stimulated Fas targets activated endothelium for destruction by anti-angiogenic thrombospondin-1 and pigment epithelium-derived factor. *Nature medicine*. 2002; 8(4):349–357.
97. Malchiodi-Albedi F, et al. PEDF (pigment epithelium-derived factor) promotes increase and maturation of pigment granules in pigment epithelial cells in neonatal albino rat retinal cultures. *International journal of developmental neuroscience*. 1998; 16(5):423–432. [PubMed: 9829178]
98. Sparrow JR, Boulton M. RPE lipofuscin and its role in retinal pathobiology. *Exp Eye Res*. 2005; 80(5):595–606. [PubMed: 15862166]
99. Mustafi D, et al. Photoreceptor phagocytosis is mediated by phosphoinositide signaling. *The FASEB Journal*. 2013; 27(11):4585–4595. [PubMed: 23913857]
100. Zhou T, et al. The pathogenic role of the canonical Wnt pathway in age-related macular degeneration. *Investigative ophthalmology & visual science*. 2010; 51(9):4371–4379. [PubMed: 19875668]
101. Dong A, et al. Oxidative stress promotes ocular neovascularization. *Journal of cellular physiology*. 2009; 219(3):544–552. [PubMed: 19142872]
102. Amano S, et al. Pigment epithelium-derived factor inhibits oxidative stress-induced apoptosis and dysfunction of cultured retinal pericytes. *Microvascular research*. 2005; 69(1):45–55. [PubMed: 15797260]
103. Valnickova Z, et al. Heparin binding induces a conformational change in pigment epithelium-derived factor. *Journal of Biological Chemistry*. 2007; 282(9):6661–6667. [PubMed: 17202143]

104. Alberdi EM, Weldon JE, Becerra SP. Glycosaminoglycans in human retinoblastoma cells: heparan sulfate, a modulator of the pigment epithelium-derived factor-receptor interactions. *BMC biochemistry*. 2003; 4(1):1. [PubMed: 12625842]
105. Filleur S, et al. Characterization of PEDF: a multi-functional serpin family protein. *Journal of cellular biochemistry*. 2009; 106(5):769–775. [PubMed: 19180572]
106. Alberdi E, Aymerich MS, Becerra SP. Binding of Pigment Epithelium-derived Factor (PEDF) to Retinoblastoma Cells and Cerebellar Granule Neurons EVIDENCE FOR A PEDF RECEPTOR. *Journal of Biological Chemistry*. 1999; 274(44):31605–31612. [PubMed: 10531367]
107. Bernard A, et al. Laminin receptor involvement in the anti-angiogenic activity of pigment epithelium-derived factor. *Journal of Biological Chemistry*. 2009; 284(16):10480–10490. [PubMed: 19224861]
108. Park K, et al. Identification of a novel inhibitor of the canonical Wnt pathway. *Molecular and cellular biology*. 2011; 31(14):3038–3051. [PubMed: 21576363]
109. George SJ. Wnt pathway a new role in regulation of inflammation. *Arteriosclerosis, thrombosis, and vascular biology*. 2008; 28(3):400–402.
110. Masckauchán TNH, Kitajewski J. Wnt/Frizzled signaling in the vasculature: new angiogenic factors in sight. *Physiology*. 2006; 21(3):181–188. [PubMed: 16714476]
111. Chen Y, et al. Activation of the Wnt pathway plays a pathogenic role in diabetic retinopathy in humans and animal models. *The American journal of pathology*. 2009; 175(6):2676–2685. [PubMed: 19893025]
112. Ruegg C, et al. Antiangiogenic peptides and proteins: from experimental tools to clinical drugs. *Biochim Biophys Acta*. 2006; 1765(2):155–77. [PubMed: 16263219]
113. Haviv FBM, Kalvin DM, Schneider AJ, Davidson DJ, Majest SM, McKay LM, Haskell CJ, Bell RL, Nguyen B, Marsh KC, Surber BW, Uchic JT, Ferrero J, Wang YC, Leal J, Record RD, Hodde J, Badylak SF, Lesniewski RR, Henkin J. Thrombospondin-1–Mimetic Peptide Inhibitors of Angiogenesis and Tumor Growth: Design, Synthesis, and Optimization of Pharmacokinetics and Biological Activities. *J Med Chem*. 2005; 48(8):2838–46. [PubMed: 15828822]
114. Gologorsky D, Thanos A, Vavvas D. Therapeutic interventions against inflammatory and angiogenic mediators in proliferative diabetic retinopathy. *Mediators of inflammation*. 2012; 2012:629452. [PubMed: 23028203]
115. Hoekstra R, et al. Phase I study of the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 with 5-fluorouracil and leucovorin: a safe combination. *European journal of cancer*. 2006; 42(4):467–72. [PubMed: 16406507]
116. Punekar S, et al. Thrombospondin 1 and its mimetic peptide ABT-510 decrease angiogenesis and inflammation in a murine model of inflammatory bowel disease. *Pathobiology: journal of immunopathology, molecular and cellular biology*. 2008; 75(1):9–21.
117. Ebbinghaus S, et al. Phase 2 study of ABT-510 in patients with previously untreated advanced renal cell carcinoma. *Clinical cancer research: an official journal of the American Association for Cancer Research*. 2007; 13(22 Pt 1):6689–95. [PubMed: 18006769]
118. Reiher FK, et al. Inhibition of tumor growth by systemic treatment with thrombospondin-1 peptide mimetics. *International Journal of Cancer*. 2002; 98(5):682–689. [PubMed: 11920636]
119. Furrer JLB, Basrur V, Roberts DD, Barchi JJ Jr. Conformational analysis of an alpha3beta1 integrin-binding peptide from thrombospondin-1: implications for antiangiogenic drug design. *J Med Chem*. 2006; 49(21):6324–33. [PubMed: 17034138]
120. Garside SA, et al. A thrombospondin-mimetic peptide, ABT-898, suppresses angiogenesis and promotes follicular atresia in pre- and early-antral follicles in vivo. *Endocrinology*. 2010; 151(12):5905–5915. [PubMed: 20881256]
121. Hoekstra R, et al. Phase I Safety, Pharmacokinetic, and Pharmacodynamic Study of the Thrombospondin-1 Mimetic Angiogenesis Inhibitor ABT-510 in Patients With Advanced Cancer. *Journal of Clinical Oncology*. 2005; 23(22):5188–5197. [PubMed: 16051960]
122. Baker LH, et al. Randomized, phase II study of the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 in patients with advanced soft tissue sarcoma. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2008; 26(34):5583–8. [PubMed: 18981463]

123. Baker LDG, Mendelson DS, et al. A randomized phase 2 study of the thrombospondinmimetic peptide ABT-510 in patients with advanced soft tissue sarcoma (STS). *J Clin Oncol*. 2005; 23(Suppl:819s) Abstract 9013.
124. Sorbera LA, Bayes M. Drugs for the future. ABT-510. *Drugs Fut*. 2005; 30(11)
125. Gietema JA, et al. A phase I study assessing the safety and pharmacokinetics of the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 with gemcitabine and cisplatin in patients with solid tumors. *Annals of Oncology*. 2006; 17(8):1320–1327. [PubMed: 16728485]
126. Amaral J, Becerra SP. Effects of human recombinant PEDF protein and PEDF-derived peptide 34-mer on choroidal neovascularization. *Investigative ophthalmology & visual science*. 2010; 51(3):1318–1326. [PubMed: 19850839]
127. Johnston EK, Francis MK, Knepper JE. Recombinant pigment epithelium-derived factor PEDF binds vascular endothelial growth factor receptors 1 and 2. *In Vitro Cellular & Developmental Biology-Animal*. 2015; 51(7):730–738. [PubMed: 25948043]
128. Volz, K., et al. Anti-angiogenic fragments fo pigment epithelium-derived factor (pedf). *Google Patents*; 2003.
129. Seger, R., Maik-Rachline, G. Variants of pigment epithelium derived factor and uses thereof. *Google Patents*; 2012.
130. Yamagishi, S-i, et al. Method for preventing or treating malignant melanoma. *Google Patents*; 2005.
131. Yamagishi, S-i, Imaizumi, T. Method for preventing or treating osteosarcoma. *Google Patents*; 2005.
132. Bouck, NP., Dawson, DW., Gillis, PR. Methods and compositions for inhibiting angiogenesis. *Google Patents*; 2001.
133. Ho T-C, et al. PEDF induces p53-mediated apoptosis through PPAR gamma signaling in human umbilical vein endothelial cells. *Cardiovascular research*. 2007; 76(2):213–223. [PubMed: 17651710]
134. Gao D, et al. Bone marrow-derived endothelial progenitor cells contribute to the angiogenic switch in tumor growth and metastatic progression. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*. 2009; 1796(1):33–40. [PubMed: 19460418]
135. Kadoya M, et al. Pigment epithelium-derived factor inhibits the growth of human esophageal squamous cell carcinoma by suppressing neovascularization. [*Hokkaido igaku zasshi*] *The Hokkaido journal of medical science*. 2015; 90(1):17–29. [PubMed: 26118299]
136. Yang H, Grossniklaus HE. Constitutive Overexpression of Pigment Epithelium–Derived Factor Inhibition of Ocular Melanoma Growth and Metastasis. *Investigative ophthalmology & visual science*. 2010; 51(1):28–34. [PubMed: 19661223]
137. Stellmach V, et al. Prevention of ischemia-induced retinopathy by the natural ocular antiangiogenic agent pigment epithelium-derived factor. *Proceedings of the National Academy of Sciences*. 2001; 98(5):2593–2597.
138. Mori K, et al. Regression of ocular neovascularization in response to increased expression of pigment epithelium–derived factor. *Investigative ophthalmology & visual science*. 2002; 43(7): 2428–2434. [PubMed: 12091447]
139. Rasmussen H, et al. Clinical protocol. An open-label, phase I, single administration, dose-escalation study of ADGVPEDF. 11D (ADPEDF) in neovascular age-related macular degeneration (AMD). *Human gene therapy*. 2001; 12(16):2029–2032. [PubMed: 11727737]
140. Campochiaro PA, et al. Adenoviral vector-delivered pigment epithelium-derived factor for neovascular age-related macular degeneration: results of a phase I clinical trial. *Human gene therapy*. 2006; 17(2):167–176. [PubMed: 16454650]
141. Mirochnik Y, et al. SHORT PEDF-DERIVED PEPTIDE INHIBITS ANGIOGENESIS AND TUMOR GROWTH. *Clinical cancer research: an official journal of the American Association for Cancer Research*. 2009; 15(5):1655–1663. [PubMed: 19223494]
142. Filleur S, et al. Two Functional Epitopes of Pigment Epithelial–Derived Factor Block Angiogenesis and Induce Differentiation in Prostate Cancer. *Cancer Research*. 2005; 65(12): 5144–5152. [PubMed: 15958558]

143. Gao X, et al. PEDF and PEDF-derived peptide 44mer protect cardiomyocytes against hypoxia-induced apoptosis and necroptosis via anti-oxidative effect. *Scientific reports*. 2014;4.
144. Longeras R, et al. A PEDF-Derived Peptide Inhibits Retinal Neovascularization and Blocks Mobilization of Bone Marrow-Derived Endothelial Progenitor Cells. *Experimental Diabetes Research*. 2012; 2012:518426. [PubMed: 21754923]
145. Famiglietti EV, et al. Immunocytochemical localization of vascular endothelial growth factor in neurons and glial cells of human retina. *Brain Research*. 2003; 969(1–2):195–204. [PubMed: 12676380]
146. Nishijima K, et al. Vascular Endothelial Growth Factor-A Is a Survival Factor for Retinal Neurons and a Critical Neuroprotectant during the Adaptive Response to Ischemic Injury. *The American Journal of Pathology*. 2007; 171(1):53–67. [PubMed: 17591953]
147. Eremina V, et al. VEGF Inhibition and Renal Thrombotic Microangiopathy. *New England Journal of Medicine*. 2008; 358(11):1129–1136. [PubMed: 18337603]
148. Walia A, et al. Endostatin's emerging roles in angiogenesis, lymphangiogenesis, disease, and clinical applications. *Biochimica et Biophysica Acta (BBA)-General Subjects*. 2015; 1850(12):2422–2438. [PubMed: 26367079]
149. Poluzzi C, Iozzo RV, Schaefer L. Endostatin and endorepellin: A common route of action for similar angiostatic cancer avengers. *Advanced drug delivery reviews*. 2016; 97:156–173. [PubMed: 26518982]
150. Nguyen TMB, et al. Endostatin induces autophagy in endothelial cells by modulating Beclin 1 and β -catenin levels. *Journal of cellular and molecular medicine*. 2009; 13(9b):3687–3698. [PubMed: 19298526]
151. Sudhakar A, et al. Human tumstatin and human endostatin exhibit distinct antiangiogenic activities mediated by $\alpha v\beta 3$ and $\alpha 5\beta 1$ integrins. *Proceedings of the national academy of sciences*. 2003; 100(8):4766–4771.
152. Hajitou A, et al. The antitumoral effect of endostatin and angiostatin is associated with a down-regulation of vascular endothelial growth factor expression in tumor cells. *The FASEB journal*. 2002; 16(13):1802–1804. [PubMed: 12354694]
153. Kim Y-M, et al. Endostatin blocks vascular endothelial growth factor-mediated signaling via direct interaction with KDR/Flk-1. *Journal of biological chemistry*. 2002; 277(31):27872–27879. [PubMed: 12029087]
154. Karumanchi SA, et al. Cell surface glypicans are low-affinity endostatin receptors. *Molecular cell*. 2001; 7(4):811–822. [PubMed: 11336704]
155. Deryugina EI, Quigley JP. Tumor angiogenesis: MMP-mediated induction of intravasation-and metastasis-sustaining neovasculature. *Matrix Biology*. 2015; 44:94–112. [PubMed: 25912949]
156. Shay G, Lynch CC, Fingleton B. Moving targets: emerging roles for MMPs in cancer progression and metastasis. *Matrix Biology*. 2015; 44:200–206. [PubMed: 25652204]
157. Wickström SA, Alitalo K, Keski-Oja J. Endostatin associates with integrin $\alpha 5\beta 1$ and caveolin-1, and activates Src via a tyrosyl phosphatase-dependent pathway in human endothelial cells. *Cancer research*. 2002; 62(19):5580–5589. [PubMed: 12359771]
158. Wickström SA, Alitalo K, Keski-Oja J. Endostatin associates with lipid rafts and induces reorganization of the actin cytoskeleton via down-regulation of RhoA activity. *Journal of Biological Chemistry*. 2003; 278(39):37895–37901. [PubMed: 12851410]
159. SHICHIRI M, YHIRATA. Antiangiogenesis signals by endostatin. *The FASEB Journal*. 2001; 15(6):1044–1053. [PubMed: 11292666]
160. Wickström SA, et al. Endostatin-induced modulation of plasminogen activation with concomitant loss of focal adhesions and actin stress fibers in cultured human endothelial cells. *Cancer research*. 2001; 61(17):6511–6516. [PubMed: 11522648]
161. Hanai, J-i, et al. Endostatin is a potential inhibitor of Wnt signaling. *The Journal of cell biology*. 2002; 158(3):529–539. [PubMed: 12147676]
162. Damico R, et al. Serum endostatin is a genetically determined predictor of survival in pulmonary arterial hypertension. *American journal of respiratory and critical care medicine*. 2015; 191(2):208–218. [PubMed: 25489667]

163. Sodha NR, et al. Endostatin and angiostatin are increased in diabetic patients with coronary artery disease and associated with impaired coronary collateral formation. *American Journal of Physiology-Heart and Circulatory Physiology*. 2009; 296(2):H428–H434. [PubMed: 19074676]
164. Available at <https://clinicaltrials.gov/ct2/show/NCT01301443?term=angiostatin&rank=4>.
165. Cao, Y., Xue, L. *Seminars in thrombosis and hemostasis*. Copyright© 2004 by Thieme Medical Publishers, Inc; 333 Seventh Avenue, New York, NY 10001, USA: 2004. Angiostatin.
166. Magnusson S, et al. The primary structure of prothrombin, the role of vitamin K in blood coagulation and a thrombin-catalyzed ‘negative feed-back’ control mechanism for limiting the activation of prothrombin. *Prothrombin and related coagulation factors*. 1975:25–46.
167. Griscelli F, et al. Angiostatin gene transfer: inhibition of tumor growth in vivo by blockage of endothelial cell proliferation associated with a mitosis arrest. *Proceedings of the National Academy of Sciences*. 1998; 95(11):6367–6372.
168. Li F, et al. Human glioma cell BT325 expresses a proteinase that converts human plasminogen to kringle 1–5-containing fragments. *Biochemical and biophysical research communications*. 2000; 278(3):821–825. [PubMed: 11095991]
169. Moser TL, et al. Endothelial cell surface F1-FO ATP synthase is active in ATP synthesis and is inhibited by angiostatin. *Proceedings of the National Academy of Sciences*. 2001; 98(12):6656–6661.
170. Moser TL, et al. Angiostatin binds ATP synthase on the surface of human endothelial cells. *Proceedings of the National Academy of Sciences*. 1999; 96(6):2811–2816.
171. Troyanovsky B, et al. Angiomotin an angiostatin binding protein that regulates endothelial cell migration and tube formation. *The Journal of cell biology*. 2001; 152(6):1247–1254. [PubMed: 11257124]
172. Available at <https://clinicaltrials.gov/ct2/results?term=angiostatin>.
173. Tolentino M. Systemic and ocular safety of intravitreal anti-VEGF therapies for ocular neovascular disease. *Survey of ophthalmology*. 2011; 56(2):95–113. [PubMed: 21335144]
174. Meyer CH, et al. Incidence of rhegmatogenous retinal detachments after intravitreal antivasular endothelial factor injections. *Acta ophthalmologica*. 2011; 89(1):70–75. [PubMed: 21176118]
175. Bakri S, et al. Immediate intraocular pressure changes following intravitreal injections of triamcinolone, pegaptanib, and bevacizumab. *Eye*. 2009; 23(1):181–185. [PubMed: 17693999]
176. Gismondi M, et al. Short-term effect of intravitreal injection of Ranibizumab (Lucentis) on intraocular pressure. *Journal of glaucoma*. 2009; 18(9):658–661. [PubMed: 20010243]
177. Hoang QV, et al. Effect on intraocular pressure in patients receiving unilateral intravitreal anti-vascular endothelial growth factor injections. *Ophthalmology*. 2012; 119(2):321–326. [PubMed: 22054994]
178. Brouzas D, et al. Massive choroidal hemorrhage after intravitreal administration of bevacizumab (Avastin) for AMD followed by contralateral sympathetic ophthalmia. *Clin Ophthalmol*. 2009; 3:457–459. [PubMed: 19714263]
179. Ladas ID, et al. Safety of repeat intravitreal injections of bevacizumab versus ranibizumab: our experience after 2,000 injections. *Retina*. 2009; 29(3):313–318. [PubMed: 19287287]
180. Chang LK, et al. RPE tears after pegaptanib treatment in age-related macular degeneration. *Retina*. 2007; 27(7):857–863. [PubMed: 17891009]
181. Smith BT, Kraus CL, Apte RS. Retinal pigment epithelial tears in ranibizumab-treated eyes. *Retina*. 2009; 29(3):335–339. [PubMed: 19174716]
182. Shah CP, et al. Retinal Pigment Epithelial Tear After Intravitreal Bevacizumab Injection. *American Journal of Ophthalmology*. 2006; 142(6):1070–1071.e1. [PubMed: 17157598]
183. Wang S, Sorenson CM, Sheibani N. Lack of thrombospondin 1 and exacerbation of choroidal neovascularization. *Archives of Ophthalmology*. 2012; 130(5):615–20. [PubMed: 22232368]
184. Mac Nair CE, et al. Retinal glial responses to optic nerve crush are attenuated in Bax-deficient mice and modulated by purinergic signaling pathways. *J Neuroinflammation*. 2016; 13(1):93. [PubMed: 27126275]
185. Li Y, Schlamp CL, Nickells RW. Experimental induction of retinal ganglion cell death in adult mice. *Invest Ophthalmol Vis Sci*. 1999; 40(5):1004–8. [PubMed: 10102300]

186. Schlamp CL, et al. Evaluation of the percentage of ganglion cells in the ganglion cell layer of the rodent retina. *Mol Vis.* 2013; 19:1387–96. [PubMed: 23825918]

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Figure 1.

Factors associated with pathogenesis of AMD and CNV. A) Dysregulated production of angioregulatory factors will affect various aspects of angiogenesis contributing to vascular dysfunction and proliferation. B) The organization of RPE-choroid complex under normal conditions (top panel), and its disorganization in AMD and development of choroidal neovascularization (lower panel).

Figure 2.
Domain organization of TSP1 and function of TSP1-derived peptides from these domains.

Figure 3.
Domain organization of PEDF and function of PEDF derived peptides from these domains.

Figure 4.

PEDF deficiency minimally affects choroidal neovascularization. A) *Serpinf1* +/+ and *Serpinf1* -/- mice (6-weeks of age; female) were subjected to laser-induced photocoagulation. The area of neovascularization was assessed as previously described [183]. B) The quantitative assessment of data. Please note no significant difference in areas of neovascularization in *Serpinf1* -/- mice compared with *Serpinf1* +/+ mice ($P > 0.05$; $n=20$ eyes). Bar=50 μm .

Figure 5.

PEDF deficiency does not exacerbate loss of retinal ganglion cells in response to nerve crush. *Serpinf1* +/+ and *Serpinf1* -/- mice (8 weeks of age; male and female) were subjected to nerve crush procedure (Crush) or procedure without crush (control) as described previously [184, 185]. Animals were sacrificed two-weeks later and retinal flat mounts were evaluated for number of BRN3A (brain-specific homeobox/POU domain protein 3A) positive cells per 100 μm^2 [186]. A) Representative images of retinal flatmounts stained with BRN3A antibody. B) The mean number of BRN3A positive cells per 100 μm^2 . Please note the dramatic decrease in cell number following nerve crush in *Serpinf1* +/+ and *Serpinf1* -/- mice. The differences between *Serpinf1* +/+ and *Serpinf1* -/- mice were not significant ($P > 0.05$; $n=8$). C) The mean percentage of cells remaining, relative to control. There was no significant difference in percentage of cells remaining in *Serpinf1* +/+ compared with *Serpinf1* -/- mice ($P > 0.05$; $n=8$).

Table 1

Impacts of TSP1 expression on function of various retinal cell types.

Cell characteristic	<i>Thbs1</i> -/- REC	<i>Thbs1</i> -/- ChEC	<i>Thbs1</i> -/- RPE	<i>Thbs1</i> -/- RPC
Morphology	Normal	Spindly morphology	Normal	Normal
Specific markers		No significant difference in VE-CAD and PECAM-1	Decreased RPE65 expression	No significant difference
Proliferation	Increased	Decreased	Increased	Decreased
Apoptosis basal	Decreased pro-apoptotic signaling	Increased	No difference	
Challenged conditions		Increased	Increased	
Migration	Increased	Decreased	Decreased	Decreased
Adhesion		Less adherent to FN, VN, Col I and Col IV	More adherent to Col I and Col IV	Less adherent to FN, VN More adherent to Col I
Junctional protein localization		No significant impact on junctional localization	More ZO1 nuclear localization	
Oxidative stress			Increased	
VEGF expression		No difference	Increased	
Capillary morphogenesis		Decreased	NA	NA
Phagocytosis	NA	NA	Decreased	NA

Table 2

Impacts of PEDF expression on function of various retinal cell types.

Cell characteristic	<i>Serpinf1</i> -/- REC	<i>Serpinf1</i> -/- ChEC	<i>Serpinf1</i> -/- RPE
Morphology	Normal	Normal	Abnormal (elongated cells)
Specific markers	Decreased PECAM-1	Decreased VE-CAD and PECAM-1	Decreased bestrophin expression
Proliferation	Increased	Increased	Increased
Apoptosis basal	No difference	No difference	No difference
Challenged conditions	Increased	Increased	Increased
Migration	Decreased	Decreased	Decreased
Adhesion	Less adherent to FN, VN, Col I and Col IV	Less adherent to FN, VN, Col I and Col IV	More adherent to FN and VN
Junctional protein localization	No significant impact on junctional localization	No significant impact on junctional localization	Loss of N-cadherin localization
Oxidative stress	Increased	Increased	Increased
VEGF expression	Decreased	Increased	Decreased
Capillary morphogenesis	Decreased	Decreased	NA
Phagocytosis	NA	NA	Increased