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Regulation of Angiogenesis in Choroidal Neovascularization of Age Related Macular Degeneration by Endogenous Angioinhibitors

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1. Introduction

The sense of vision has utmost significance and the loss of vision leads to the impairment of active human behavior as evident in pathological disorders that affect vision. Among different pathological visual disorders, Age Related Macular Degeneration (AMD/ARMD) is of serious concern as a leading cause of blindness, observed with aging globally. The clinical manifestation of AMD includes retinal damage with the degeneration of macula, leading to the partial or complete loss of acuity in vision. One form of pathologic AMD named, "wet form of AMD", involves the growth of new blood vessels from the choroid which lies underneath the retina, leading to the pathological blood vessel growth termed as Choroidal Neovascularization (CNV), with subsequent damage to the retina. Thus, choroidal neovascularization reflects a pathological angiogenic condition, where the loss of regulation over angiogenesis leads to the retinal damage. It also indicates that, the regulation of pathological angiogenesis can be an efficient strategy in preventing CNV of AMD. Though, some genetic disposition and aging factors are identified as peculiar etiological factors causing AMD; recent studies have shown that different cellular mechanisms regulating angiogenesis are common in different angiogenic scenarios including CNV. Further, the role of different endogenous angiogenesis inhibitors/angioinhibitors conferring the tissues with angiogenic regulation has been deciphered, which can be applied for regulation of CNV in AMD through inhibition of angiogenic signaling mechanisms. The present chapter provides an overview of the role of factors leading to choroidal neovascularization, the mechanisms underlying such angiogenesis and also the scope for endogenous angioinhibitors in regulation of CNV of AMD.

1.1 Retina and choroid

Retina is the inner most layer of the eye, which possesses anatomically ten distinct layers that are broadly categorized into two layers. The inner neural layer comprising of extensive

nervous tissue towards the vitreous chamber and the outer retinal pigmented epithelium (RPE) adhering to the choroid. Some of the functions of the RPE include the phagocytosis of outer retinal segmental discs, maintenance of chorio-capillaries, fluid and electrolyte balance in subretinal space. Choroid is the highly vascular and pigmented tissue of the eye lying between the retina and the sclera. It consists of lamina suprachoroidea adhering to sclera, followed by lamina vesculosa, chorio-capillaries, stroma and Bruch membrane adhering to the RPE. Choroid is rich in vasculature and the extracellular matrix (ECM) components, including collagen and elastin fibers. It provides nutrient, metabolite and gaseous exchange to the retina by diffusion through chorio-capillaries.

1.2 Choroidal neovascularization in age related macular degeneration

The histological proximity between retinal pigmented epithelium and choroid confers not only physiological but also pathological effect on RPE. The mechanical barrier that separates the RPE from choroid is the Bruch membrane, which in turn consists of basement membrane secreted by RPE, inner collagenous layer, elastic layer, outer collagenous zone and the basement membrane of chorio-capillaries acting as a mechanical barrier for the underlying chorio-capillaries, but facilitating diffusion of metabolites and gaseous exchange for RPE. In cases of CNV the Bruch membrane is distorted with initial deposition of lipid and protienaceous component called 'drusen' followed by the growth and penetration of blood capillaries from choroid into Bruch membrane, finally leading to the leakage of fluid into sub-retinal spaces and retinal or retinal pigmented epithelial damage (Green, 1999; Green and Enger, 1993; Jager et al., 2008).

1.3 Factors for choroidal neovascularization in age related macular degeneration

Pathological neovascularization in CNV of AMD is considered to be contributed by both the angiogenesis and vasculogenesis, which are the processes of *de-novo* blood vessel formation (Chan-Ling et al., 2011; Jager et al., 2008). Angiogenesis is the process of formation of new blood vessels from the pre-existing ones, which involves the role of different cell types and remodeling of ECM. The inception of different cell types involved in the angiogenesis, such as, the endothelial cells (ECs) of RPE and choroid involved in CNV, mural cells and inflammatory cells occurs through vasculogenesis, by the differentiation of endothelial progenitor cells (EPCs). The EPCs found in the normal circulation are recruited into angiogenic sites, where they differentiate into different cell types leading to angiogenesis (Chan-Ling et al., 2011; Jager et al., 2008). However, the salient feature of neovascularization involves the common sequential events of angiogenesis including the proliferation of ECs, degradation of ECM or vascular basement membrane (VBM) by ECs through secretion of proteases, migration and differentiation of ECs into tip and stalk cells, lumen development, ECM reorganization and finally vessel anastomosing into functional capillaries (Carmeliet and Jain, 2000). These sequential steps of angiogenesis are considered to be common for CNV, which are initiated by the release of angiogenic factors by the RPE and other cell types differentiated from EPCs or infiltrating through the leaky capillaries in response to aging evoked stress (Alon et al., 1995; Grossniklaus et al., 2002). The initiating cellular and physiological factors that lead to the secretion of angiogenic factors by ECs and other cell types have been identified in different studies, which can be systematically framed for synergistic interpretation of etiological factors leading to CNV.

The normal function of phagocytosis and degradation of phagocytosed membranes is impaired with aging in RPE, leading to the accumulation of lipofuscin in these cells, with senescence (Marshall, 1987; Young and Bok, 1969). Ischemia and hypoxia evident in the ocular tissues of CNV are identified as factors promoting free radical generation in RPE and also the release of cellular lipids and proteinaceous deposits into the Bruch membrane (Spaide et al., 2003). Thus, impairing Bruch membrane's barrier function and in turn leading to the secretion of different angiogenic factors like vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF- β), basic fibroblast growth factor (bFGF), insulin-like growth factor-1 and platelet derived growth factor (PDGF) by the RPE and the macrophages and stromal cells that are recruited by the differentiation of EPCs (Alon et al., 1995; Grossniklaus et al., 2002; Lu and Adamis, 2006; Penn et al., 2008; Young and Bok, 1969). Damage to the Bruch membrane is considered to enhance the diffusion of the growth factors, which elicit angiogenic signaling in the ECs (Lu and Adamis, 2006; Marshall, 1987; Penn et al., 2008).

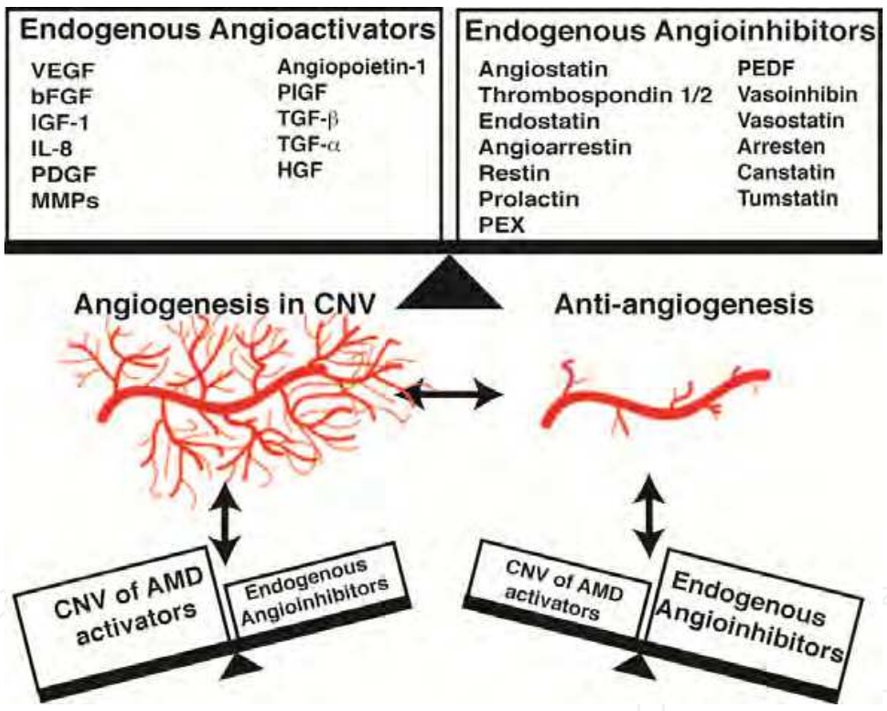

Fig. 1. **The angiogenic balance between endogenous angioactivators and angioinhibitors regulate vascular homeostasis.** Angiogenesis under physiological and pathological conditions is associated with up-regulation of endogenous angioactivators and/or down-regulation of endogenous angioinhibitors. Up-regulation of angioinhibitors and/or down-regulation of angioactivators may be associated with impaired neovascularization capacity in the choroidal neovascularization in age related macular degeneration (CNV of AMD). VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; IGF-I, insulin-like growth factor-I; IL-8, interleukin-8; PDGF, platelet-derived growth factor; PlGF, placental growth factor; TGF-α and β, transforming growth factor-α and β;
HGF, Hepatocyte growth factor

Endogenous Angioactivators	Potent Receptors	Angiogenic action
Vascular endothelial growth factor (VEGF) & Placental growth factor (PlGF)	VEGFRs (Flt-1, Flk-1, KDR, Flt-4), Neuropilins, HSPG, integrins	Increases EC permeability, proliferation, migration, NO, uPA/PAI-1 & MMP production Inhibiting EC apoptosis, Promotes ECM degradation Monocyte migration
Transforming growth factor-β (TGF-α, –β)	Transforming growth factor receptors	Increased vessel stability and organization, promote secretion of ECM components
basic Fibroblast growth factor (bFGF)	FGFRs, HSPG, integrins	Promotes EC proliferation, migration, tube formation, ECM degradation, vessel maturation
Insulin-like growth factor-1 (IGF-1)	Insulin-like growth factor receptors	Promotes EC migration, proliferation, tube formation
Platelet derived growth factor (PDGF)	PDGF-α, –β, GPCRs, integrins	Increases EC permeability, proliferation, migration
Angiopoietin-1	Tie-2, integrins	EC sprouting, Vessel stabilization
Hepatocyte growth factor (HGF)	Hepatocyte growth factor receptor	Promotes tubulogenesis along with other factors
Interleukin-8 (IL-8)	C-X-C chemokine receptor type (CXCR-1,2)	Activates neovascularization increasing invasiveness of different cell types
Matrix metalloproteinases (MMPs)		Degradation of ECM components promoting EC migration and vessel organization, release of ECM or cell surface bound/sequestred angiogenic fcators

Table 1. Endogenous activators, their receptors and angiogenic activities (EC: endothelial cell, ECM: extracellular matrix, FGFRs: Fibroblast growth factor receptors, Flk-1: Fetal liver kinase-1, Flt-1, 4: fms-related tyrosine kinase, GPCRs: G-protein coupled receptors, HSPG: Heparan sulfate proteoglycan, KDR: kinase insert domain receptor, MMP: matrix metallo proteinase, NO: nitric oxide, Pak: p21 protein activated kinase, PDGF: platelet derived growth factor, Tie: tyrosine kinase with immunoglobulin-like and EGF-like domains, VEGFRs: vascular endothelial growth factor receptors)

The integrins and other ECM binding receptors present on ECs are essential in maintaining the ECM promoted survival and migration in angiogenesis (Avraamides et al., 2008; Mettouchi and Meneguzzi, 2006). The synergistic activation of integrins and other ECM binding receptors on ECs by the growth factors and cytokines leads to the activation of different signaling cascades mediated by the kinases, secondary messengers, transcription factors such as, nuclear factor kappa β (NF-κβ), hypoxia inducible factor-1α (HIF-1α), and other enzymes such as, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and metalloproteinases (MMPs) (Avraamides et al., 2008; Boosani et al., 2007; Egeblad and Werb, 2002; Mettouchi and Meneguzzi, 2006; Oklu et al., 2010). The transcription factors that are stabilized, up-regulated or expressed under hypoxia also lead to activation of different signaling cascades that promote effective survival and proliferation of ECs. The secretion of proteases such as matrix metallo-proteinases (MMPs) including collagenases and elastases, which degrade the collagen and elastin of vascular basement membrane (VBM) promote the migration of ECs. The urokinase is another proteinase, which binds to its receptors (urikanse binding receptor, uPAR) and activates signaling cascades leading to the secretion of MMPs, which promote migration of ECs and angiogenesis. The organization and differentiation of

migrating ECs into tip and stalk cells is further enumerated to be regulated by Wingless type (Wnt)/Frizzled-Notch signaling that provides an insight about formation of functional capillaries in neovascular vessels (Dejana, 2010; Zerlin et al., 2008).

The inflammatory cells that are recruited through the expression of cytokines such as monocyte chemo-attractant protein-1 (Ccl2/MCP-1), Chemokine (C-X-C motif) liagnd 1 (CXCL1), macrophage inflammatory protein-1/-2 (MIP-1, MIP-2) are also considered to play role in CNV progression (Hendricks, 2006). Further, the intriguing stimulative role of Bruch membrane in promoting AMD is also being deciphered by identifying the complement components 3a and 5a (C3a and C5a), which lead to the up-regulation of VEGF-A (Nozaki et al., 2006). Thus, the orchestration of various signaling events at different stages of angiogenesis leads to the neovascularization. The angiogenic ECs lining the neovascular vessels arising due to the above factors in CNV are found to possess fenestrations and also organize into defective capillaries leading to the leakage of macromolecules as well as vascular cells into the Bruch membrane and sub-retinal spaces leading to the degeneration of macula of retina (Dvorak et al., 1995; Roberts and Palade, 1995).

2. Endogenous angioinhibitors

In addition to the angiogenic factors, which activate angiogenesis, tissues and ECM also possess angioinhibitors, which have the potency to inhibit the angiogenesis and thus, regulating the pathological angiogenesis by inhibiting the signaling mechanisms activated by angiogenic factors (Boosani et al., 2010; Sudhakar and Kalluri, 2010, Zhang and Ma, 2007). Nearly, 40 endogenous angioinhibitors have been characterized and some of them are found in the ocular tissues or secreted into vasculature and released into ocular tissues, where they exhibit angio-inhibition and finally regulation of CNV (Boosani et al., 2011; Sudhakar and Kalluri, 2010). The significance of imbalance in the levels of endogenous angioinhibitors and angioactivators in regulation of vascular homeostasis can be summarized as in Figure 1. This significance was also ascertained by the evaluations showing the correlation between the decrease in specific angioinhibitors and the progression of CNV (Bhutto et al., 2008).

2.1 Mechanisms of regulation of CNV by endogenous angioinhibitors

2.1.1 Vasoinhibins

The vasoinhibins (14-18 kDa) are antiangiogenic peptides found in the pituitary, retina and extrapituitary tissues. They constitute the amino terminal regions of three different precursors; prolactin, growth hormone and placental lactogen. Though their precursors do not exhibit angioinhibitory activities; vasoinhibins found in the tissues or those expressed using recombinant methods exhibit antiangiogenic properties (Clapp et al., 2008). The therapeutic potential of vasoinhibins in regulating angiogenesis in CNV and tumor growth was evaluated and studies indicate that adenovirus mediated expression of vasoinhibins inhibits CNV, *in-vivo* and also angiogenesis (Zhou et al., 2010). Mechanisms of regulation of EC survival, proliferation and migration by the vasoinhibins have been deciphered in different studies; nevertheless, the receptors through which the mechanisms are mediated still remain enigmatic. Vasoinhibins regulate the EC migration and survival through inhibition of VEGF and bFGF stimulated MAPK activation (D'Angelo et al., 1995).

Endogenous Angioinhibitor	Parent molecule	Receptors	Mode of action/ Inhibition pathways
Vasoinhibins	Prolactin, growth hormone	Not known	Sos/Ras/MAPK or eNOS /Raf/MAPK, Ca2+/ eNOS/protein phosphatase 2, Ras/Tiam-1/Rac1/Pak1, Bcl-XL, NF-kβ, caspases
PEDF	PEDF	Not known	Possible apoptosis
Arresten	Collagen IV, α1 NC1	α1β1 integrin, HSPG	Raf/MEK/ERK1/2/p38-MAPK, HIF-1α, MMPs
Canstatin	Collagen IV, α2 NC1	αVβ3, αVβ5 integrins, Fas	procaspse-8 and -9, Akt/ FAK/mToR, eIF-4EBP-1, Ribosomal S6-kinase
Tumstatin	Collagen IV, α3NC1	CD47/IAP, αVβ3, α6β1 integrins	FAK/Akt/PI3K/mTOR/ eIF-4EBP1/NFκB, COX-2 signaling
Endostatin	Collagen XVIII-NC1	αVβ1/α5β1 integrins, HSP, glypican, caveolin-1	Ras/Raf/KDR/Flk-1 / ERK/p38-MAPK/p125 FAK/HIF1α/Ephrin/TNFα/NFκB, Wnt signaling
Angiostatin	Plasminogen	ATP synthases, αVβ3 integrin, angiomotin	αVβ3 integrin mediated apoptotis, ATP synthase
Thrombospondins	TSP	CD36, IAP, CD47, HSPG, α3β1 , other integrins	Src-family kinases/ Caspase-3/p38 MAPK, TGF-β signaling
Endorepellin	Perlecan	α2β1 integrins, lipid rafts, caveolin	cAMP-PKA/FAK/p38-MAPK/Hsp27, SHP-1, Ca2+ signaling

Table 2. Endogenous angioinhibitors, their precursors, cell surface receptors and mode of action AMD/ARMD: Age related macular degeneration, Akt: protein kinase B, Bcl-XL: B-cell lymphoma-extra large, bFGF: basic fibroblast growth factor, Ccl2/MCP-1: chemoattractant protein-1, CD(CD47, CD36): cluster of differentiation, CNV: choroidal neovascularization, COX-2: cyclooxygenase-2, eIF-4EBP-1: eukaryotic translation initiation factor-4E binding protein-1, eNOS: endothelial nitric oxide synthase, ECs: endothelial cells, ECM: extracellular matrix, EPCs: endothelail progenitor cells, ERK1/2: extracellular signal-regulated kinase1/2, FAK: focal adhesion kinase, Flk-1: fetal liver kinase-1, HIF-1α: hypoxia inducible factor-1α, Hsp: heat shock protein, HSPG: Heparan sulfate proteoglycan, IAP: integrin associated protein, KDR: kinase insert domain receptor, MAPK: Mitogen activated protein kinase, MEK: MAPK-ERK kinase, MMPs: matrix metallo proteinases, mToR: mammalian target of rapamycin, NF-kβ: nuclear factor kappa β, Pak: p21 protein activated kinase, PDGF: platelet derived growth factor, PEDF: Pigment epithelium derived factor, PEX: noncatalytic Carboxy-terminal hemopexin-like domain of MMP, PI3K: phosphatidyl inositol 3-kinase, Rac: Ras-related C3 botulinin toxin susbtrate 1, Raf: Ras activated factor, Ras: Rat sarcoma, RPE: retinal pigmented epithelium, SHP: Src homology region 2 domain-conatining phopshatase, Sos: Son of sevenless, Src: Schmidt-Ruppin A-2 sarcoma viral oncogene homolog, Tiam: T-lymphoma invasion and metastasis-inducing protein, TGF-β: transforming growth factor β, TNFα: tumor necrosis factorα, TSP: thrombospondin, VBM: vascular basement membrane, VEGF: vascular endothelial growth factor, Wnt: wingless-type

VEGF activated Sos/Ras/MAPK or eNOS/Raf/MAPK-mediated proliferative signaling and Ca2+/eNOS/protein phosphatase-2 mediated vascular permeability and vasodilatation were shown to be inhibited by the vasoinhibins (Gonzalez et al., 2004; Ziche and Morbidelli, 2000). In addition vasoinhibins also inhibit the migration of EC stimulated by IL-1β through Ras/Tiam-1/Rac-1/Pak1 and promote apoptosis through conversion of Bcl-XL to proapoptoctic Bcl-Xs and NF-kβ mediated activation of initiator and effector caspases (Martini et al., 2000; Tabruyn et al., 2003).

2.1.2 Pigment Epithelium Derived Factor (PEDF)

Pigment epithelium derived factor (PEDF) is a 50 kDa, secreted, serpin family glycoprotein, first identified from the cultured fetal RPE conditioned media (Tombran-Tink et al., 1991). PEDF accumulates in the vitreous humor and is also expressed in different adult tissues (Tombran-Tink et al., 1991). Addition of PEDF to the cultured HUVECs increased the number of TUNEL positive cells suggesting apoptotic mode of action of PEDF and thus, possibly preventing EC response to ischemia *in-vivo* (Ho et al., 2007). The levels of PEDF were found to be decreased in Bruch membrane with progression of AMD and concomitant increase in VEGF levels were also identified with decrease in PEDF levels (Bhutto et al., 2008). Different methods of PEDF upregulation have been applied to investigate the effect of PEDF on CNV. Intravitreous injections of adenovirus expressing the PEDF and ultrasound-microbubble technique of noninvasive gene transfer of PEDF gene in rats exhibited significant decrease in the CNV (Gehlbach et al., 2003; Zhou et al., 2009). However, studies also demonstrate that PEDF at lower doses (90μg/ml) has negative effect on CNV whereas; higher doses (2-4 fold) can augment CNV; thus, indicating a strategic approach to be developed during clinical trials for CNV treatment with PEDF (Apte et al., 2004).

2.1.3 Angiostatin

Angiostatins are 38-45 kDa kringle domains derived by the protease activity of parent molecule plasminogen, which itself has significant role in activation of fibrinogen and blood clotting (Hayashi et al., 2008). Some of the angiostatin peptide derivates exhibit anti-angiogenic properties including inhibition of EC proliferation, tube formation and migration. The application of angiostatins in regulating CNV of AMD was evaluated by the expression of the angiostatins *in-vivo*, using viral vectors (Lai et al., 2001). Angiostatins bind to ATP synthases on the surface of ECs leading to their apoptotic death (Burwick et al., 2005; Tarui et al., 2001). Further αVβ3 integrin and angiomotin are also shown to bind angiostatin and induce apoptosis (Burwick et al., 2005; Tarui et al., 2001).

2.1.4 Thrombospondins

Thrombospondins (TSPs) are secreted ECM glycoproteins playing key role in the cellular and ECM interactions (Bornstein, 2001; Lawler, 2000). The NH2-terminal peptides derived from the TSPs, by the action of different proteases are identified to possess angioinhibitory properties. TSP-1 and TSP-2 are trimeric globular domain subunits (145 kDa) categorized into subgroup-A and subgroup-B consists of TSP's 3-5, which are pentameric subunits (110 kDa) (Bornstein, 2009). TSP-1 was the first identified ECM derived endogenous

angioinhibitor from many normal tissues and produced by a variety of cells including platelets, megakaryocytes, epithelial, endothelial and stromal cells (Bornstein, 2009). TSP-1 is secreted by the retinal-pigmented epithelium (RPE) and regulates angiogenesis in normal eye (Miyajima-Uchida et al., 2000). Immunolocalization studies showed decrease in the levels of TSP in the chorio-capillaries and the Bruch membrane of AMD samples (Bhutto et al., 2008). Wispostatin-1 (WISP-1) repeat derived peptide from TSP-1 was shown to inhibit the CNV in LASER induced CNV mice models (Cano Mdel et al., 2009). TSP-1 induces apoptosis in ECs through CD36 and integrin associated protein (IAP)/Src-family protein kinases/Caspase-3/p38 MAPK signaling (Dawson et al., 1997). In addition TSP-1 can also bind to different integrins, including CD47 and heparin sulfated proteoglycans (Kaur et al., 2011). Thus the significance of TSPs in regulation of CNV have been evaluated through detection of endogenous levels in pathological tissues and also by evaluating the effects of TSPs both *in vitro* and *in vivo.*

2.1.5 Arresten

Arresten [α1(IV)NC1], is the 26 kDa collagen type IV, α1 chain derived non-collagenous domain, which functions via binding to α1β1 integrin and heparan sulfate proteolgycans, regulating bFGF and VEGF stimulated activation of ECs (Boosani and Sudhakar, 2006; Colorado et al., 2000; Sudhakar et al., 2005). It inhibits the survival of mouse lung endothelial cells through inhibition of FAK phopshorylation in AKT independent manner (Sudhakar et al., 2005). FAK inhibition by arresten via α1β1 integrin leads to inhibition of downstream Raf/MEK/ERK1/2/p38 MAPK signaling and HIF-1α expression **(Figure 2)**. Inhibition of HIF-1α by arresten is critical in preventing hypoxic survival of ECs through VEGF regulation (Sudhakar et al., 2005). Arresten inhibited VEGF-mediated angiogenesis by promoting apoptosis, caspase-3/PARP activation and negatively impacting FAK/p38-MAPK phosphorylation, Bcl-2 and Bcl-x_L expressions leading to mouse retinal endothelial cell (MREC) death (Boosani et al., 2009). In addition angioinhibitory activity of arresten was found to inhibit bFGF induced proliferation of MREC *in-vitro* in a dose dependent manner. It also inhibited the bFGF-induced migration of MREC mediated by MMP-2 activity but not the expression levels of MMP-2 (Boosani et al., 2010). Thus, arresten was shown to effect the proliferation and migration of choroidal endothelial cells and regulate CNV of AMD. The endothelial specific inhibitory actions of arresten may be of benefit in the treatment of a variety of eye diseases with a neovascular component.

2.1.6 Canstatin

It is the 24 kDa collagen type IV, α2 derived non-collagenous domain [α2(IV)NC1], which binds to the αVβ3 and αVβ5 integrins and inhibits EC proliferation, migration and tube formation by enhancing apoptosis in these cells (Magnon et al., 2005; Magnon et al., 2007; Petitclerc et al., 2000; Roth et al., 2005). The antiangiogenic efficacy of canstatin in regulating the neovascularization of cornea was also evaluated using the recombinant canstatin in alkali burn induced neovascularization study (Lima et al., 2006; Wang et al., 2011). Cantstain was shown to induce apoptosis through the induction of Fas-ligand, activation of procaspse-8 and -9 cleavage, reduction in membrane potential, inhibition of Akt, FAK, mToR, eIF-4E/4E-BP1 and ribosomal S6-kinase phosphorylations, in cultured HUVECs **(Figure 2)** (Panka and Mier, 2003).

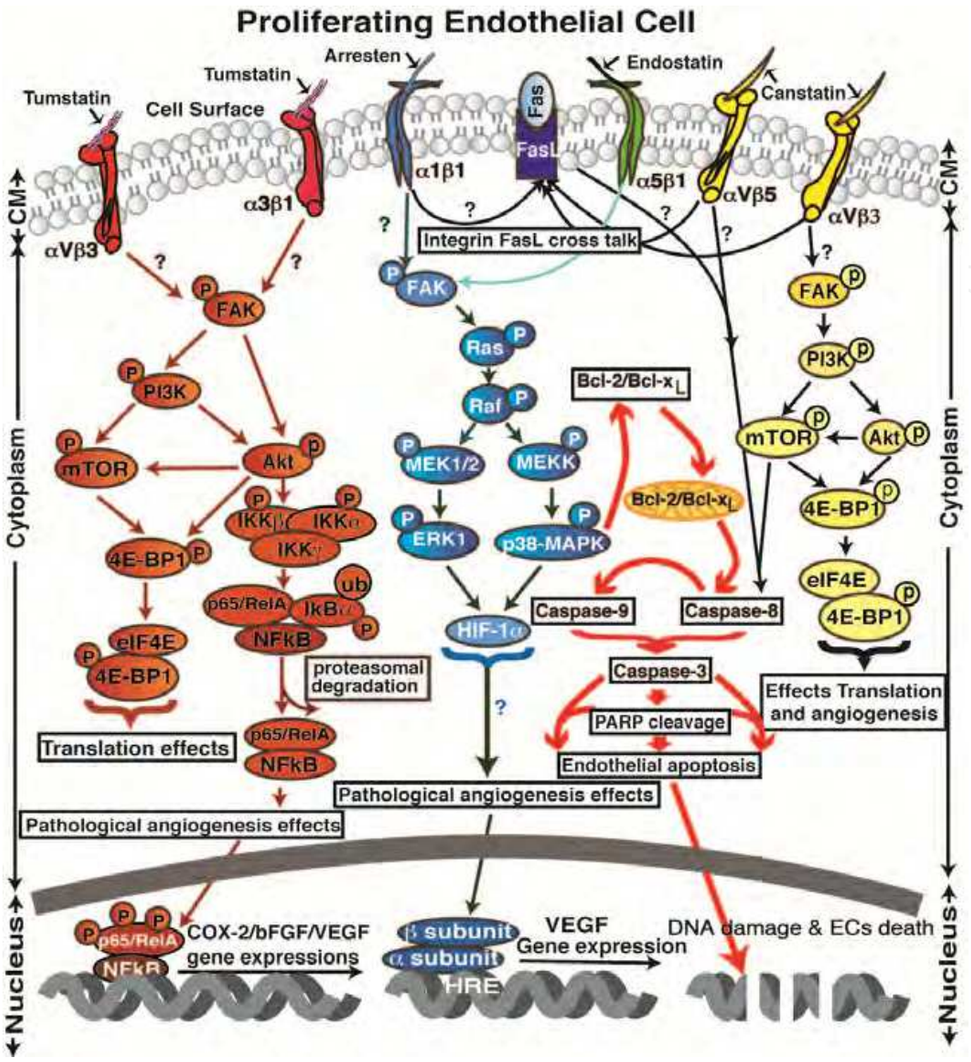

Fig. 2. **Schematic illustration of distinct angioinhibitory signaling mediated by different extracellular matrix (ECM) reloaded molecules.** Tumstatin, arresten, canstatin and endostatin interact with αVβ3/α3β1, α1β1, αVβ3/αVβ5 and α5β1 integrins respectively, to inhibit the phosphorylation of focal adhesion kinase (FAK). Tumstatin: It binds to αVβ3 and α3β1 integrins and inhibits the pathway that includes phosphorylation of FAK, PI3-K, Akt, mTOR, 4E-BP1 and eIF4E to decrease endothelial cell protein synthesis and proliferation. In addition tumstatin also inhibits NFκB mediated signaling in hypoxic conditions leading to the inhibition of COX-2, VEGF and bFGF expressions, resulting in inhibition of hypoxic tumor angiogenesis. Arresten: It binds to α1β1 integrin and inhibit phosphorylation FAK, causes inhibition of Ras, Raf, extra cellular signal related kinase 1 (ERK1) and p38 MAPK pathways that leads to inhibition of HIF-1α and VEGF expression resulting in inhibition of endothelial cell migration, proliferation and tube formation. In addition arresten initiates two apoptotic pathways, involving activation of caspase-9 and -8, leading to activation of caspase-3 and PARP cleavage. (a) Arresten activates caspase-3 directly through inhibition of FAK/p38-MAPK/Bcl-2/Bcl-x_L and activation of caspase-9; (b) Integrin α1β1 cross talk with Fas-L through mitochondrial pathway and leads to activation of caspase-8 and-3 in

proliferating endothelial cells. Canstatin: It binds to αVβ3/αVβ5 integrins and inhibits two apoptotic pathways, involving activation of caspase-8 and casoase-9, leading to activation of caspase-3. Canstatin activates procaspase-9 not only through inhibition of the FAK/PI3K/AKT pathways but also by integrins cross talking mitochondrial pathway through Fas-L dependent caspase-8 activation leads to endothelial cell apoptosis. CM represents cell membrane. Endostatin: It binds to α5β1 integrin and inhibit phosphorylation FAK, causes inhibition of Ras, Raf, extra cellular signal related kinase-1 (ERK1) and p38 MAPK pathways that leads to inhibition of endothelial cell migration and tube formation.

2.1.7 Tumstatin

Tumstatin [α3(IV)NC1], is a 28 kDa collagen type IV, α3 chain derived non-collagenous domain with anti-angiogenic and proapoptotic activities. It binds to the CD47/IAP, αVβ3, α3β1 and α6β1 integrins and inhibits the signaling cascade mediated by FAK, Akt, PI3K/mTOR/eIF-4E/4E-BP1 and NFκB/COX-2 (Boosani et al., 2007; Hamano et al., 2003; Maeshima et al., 2002; Monboisse et al., 1994; Sudhakar et al., 2003). Inhibition of eIF-4E/4E-BP1 by tumstatin leads to the regulation of cap dependent translational level of genes, whereas inhibition of transcriptional factor signaling such as NFκB leads to regulation of genes such as COX-2 at transcriptional level (Figure 2) (Boosani et al., 2007). Thus, tumstatin exhibits gene regulation in endothelail cell-specific and integrin-dependent manner. Angioinhibitory effect of tumstatin has been evaluated in regulation of CNV in mice (Boosani et al., 2011). Recombinant tumstatin regulated tube formation by mouse corneal endothelial cells (MCECs) *in-vitro* and adenoviral mediated expression of tumstatin *in-vivo* in mice has shown reduction in CNV (Boosani et al., 2011; Gunda et al., 2011).

2.1.8 Endostatin

Endostatin is the partial 20-kDa fragment of collagen type XVIII, carboxy terminal non-collagenous domain, derived from the parent collagen by proteolytic cleavage activities of elastase and cathepsin-L (Felbor et al., 2000). Endostatin is found in normal circulation enabling it to be utilized as an effective angioinhibitor without toxic effects (Fukai et al., 2002). Lower levels of endostatin have been recorded in CNV samples compared to the healthy donor eyes and within the tissues of progressive AMD (Bhutto et al., 2008; Fukai et al., 2002). Deletion of endostatin or collagen type XVIII massively up-regulates LASER induced CNV; where as administration of physiological concentrations of endostatin was able to inhibit such CNV in these mice (Marneros et al., 2007). Endostatin also down regulates the expression of VEGF in experimental CNV rat models (Takahashi et al., 2003). These observations along with the evidence of inhibition of CNV with intravenous injection of adenoviral vectors that express secretable endostatin, confirm the significance of endostatin in regulation of CNV (Mori et al., 2001; Wickstrom et al., 2003).

Endostatin elicits the anti-proliferative and anti-migratory effects by binding to different EC surface molecules and regulating the signaling cascades (Faye et al., 2009). Recombinant endostatin binds to αV integrin as shown in human endothelial cells (Rehn et al., 2001). Further studies have also shown localization of endostatin in the lipid rafts and association with caveolae (Wickstrom et al., 2002; Wickstrom et al., 2003). Surface plasmon resonance assays characterized the binding of endostatin to both αVβ1 integrins and the heparin

sulfates and also localization to the lipid rafts (Ricard-Blum et al., 2004). *In-vitro* assays using ECs also showed the co-localization of endostatin with α5β1 integrin, actin stress fibers, membrane anchor protein and caveolin-1, which enumerates the interaction of endostatin with caveolae, inhibiting EC migration through the disassembly of actin stress fibers/focal adhesions, activation of Src and impaired fibronectin deposition by ECs in response to bFGF (Wickstrom et al., 2002; Wickstrom et al., 2003; Sudhakar et al., 2003). Binding of endostatin with integrins also down-regulates the activity of RhoA-GTPase and inhibits signaling pathways mediated by small kinases of the Ras and Raf families (Ricard-Blum et al., 2004). In addition, binding to the KDR/Flk-1, endostatin inhibits the VEGF-induced tyrosine phosphorylation of KDR/Flk-1 and activation of ERK, p38 MAPK, and p125FAK in HUVECs (Kim et al., 2002; Sudhakar et al., 2003). Further signaling cascades regulated by the endostatin are being identified, which are mediated by activator protein 1 (Id), HIF1α, ephrin, tumor necrosis factor-α (TNFα), nuclear factor-κB (NFκB), coagulation cascades, adhesion molecules and Wnt, which indicate the potential role of endostatin as an endogenous angioinhibitor (Nyberg et al., 2005) **(Figure 2).**

2.2 Scope for endogenous angioinhibitors in CNV treatment

Current modalities of treatment for the CNV in AMD include the regulation of angiogenesis as angiogenesis being one of the pathological factors of neovascularization. The therapies such as LASER photocoagulation, photodynamic therapy and anti-VEGF therapies using Macugen or Lucentis, that are currently being applied to regulate the CNV have their own constraints such as development of lesions, loss of acuity in vision and frequent administration, respectively (Gallemore and Boyer, 2006). Alternative strategies for the treatment of CNV in AMD are therefore being developed in which the specific targeting on angiogenesis can be possible. Endogenous angioinhibitors are considered as one of the area to be explored in this arena to include them in regimens of complementary treatments for the regulation of CNV (Chappelow and Kaiser, 2008; Do, 2009). The signaling cascades regulated by some of endogenous angioinhibitors have been identified **(Table 2 and Figure 2),** which enabled the application of those inhibitors in CNV.

3. Conclusions

The cellular, extracellular milieu and genetic factors responsible for the neovascularization arising in AMD are being deciphered with emphasis on identifying those factors that play a key role in the inception and progression of CNV. In this scenario, different etiological factors have been identified which regulate angiogenesis, effecting both extracellular milieu and intracellular angiogenic signaling pathways. Identification of the signaling cascades leading to the pathological angiogenesis in CNV has further lead to the possibility of regulating CNV, by focusing on signaling pathways as one of the targets. Application of endogenous angioinhibitors has proven as a promising strategy in this scenario of inhibiting angiogeneic pathways that are identified in CNV. The inhibitors such as vasoinhibins, PEDF, angiostatin, endostatin, tumstatin, canstatin and arresten that have been so far evaluated for regulation of CNV have not only shown promising evidence of CNV regulation, but also provided new strategies for inhibiting CNV through differential mode of actions. Such variation exhibited by different endogenous angioinhibitors can be beneficial in targeting CNV using different combinations of these inhibitors. It can be

realized that these naturally occurring inhibitors can pose low immune reactions and thus, an efficient way of regulating diseases. Further, clinical studies using individual and combinations of endogenous angioinhibitors, included in different regimens along with current therapies of CNV would elaborate the application of endogenous angioinhibitors for regulating CNV of AMD.

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5. References

Alon, T., Hemo, I., Itin, A., Pe'er, J., Stone, J., and Keshet, E. (1995). Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. Nat Med *1*, 1024-1028.

Apte, R. S., Barreiro, R. A., Duh, E., Volpert, O., and Ferguson, T. A. (2004). Stimulation of neovascularization by the anti-angiogenic factor PEDF. Invest Ophthalmol Vis Sci *45*, 4491-4497.

Avraamides, C. J., Garmy-Susini, B., and Varner, J. A. (2008). Integrins in angiogenesis and lymphangiogenesis. Nat Rev Cancer *8*, 604-617.

Bhutto, I. A., Uno, K., Merges, C., Zhang, L., McLeod, D. S., and Lutty, G. A. (2008). Reduction of endogenous angiogenesis inhibitors in Bruch's membrane of the submacular region in eyes with age-related macular degeneration. Arch Ophthalmol *126*, 670-678.

Boosani, C. S., Gunda, V., Wang, S., Sheibani, N., and Sudhakar, A. Y. (2011). Tumstatin inhibits Choroidal Neovascularisation by Inhibiting MMP-2 activation *in-vitro* and *in-vivo.* Mol Vision. (Publication ahead of print).

Boosani, C. S., Mannam, A. P., Cosgrove, D., Silva, R., Hodivala-Dilke, K. M., Keshamouni, V. G., and Sudhakar, A. (2007). Regulation of COX-2 mediated signaling by alpha3 type IV noncollagenous domain in tumor angiogenesis. Blood *110*, 1168-1177.

Boosani, C.S., Nalabothula, N., Munugalvadla, V., Cosgrove, D., Keshamouni, V. G., Sheibani, N., and Sudhakar, A. (2009). FAK and p38-MAP kinase-dependent activation of apoptosis and caspase-3 in retinal endothelial cells by α1(IV)NC1. *Invest. Ophthalmol. Vis. Sci. 50*, 4567-4575.

Boosani, C. S., Nalabothula, N., Sheibani, N., and Sudhakar, A. (2010). Inhibitory effects of arresten on bFGF-induced proliferation, migration, and matrix metalloproteinase-2 activation in mouse retinal endothelial cells. Curr Eye Res *35*, 45-55.

Boosani, C. S., and Sudhakar, A. (2006). Cloning, purification, and characterization of a non-collagenous anti-angiogenic protein domain from human alpha1 type IV collagen expressed in Sf9 cells. Protein Expr Purif *49*, 211-218.

Bornstein, P. (2001). Thrombospondins as matricellular modulators of cell function. J Clin Invest *107*, 929-934.

Bornstein, P. (2009). Thrombospondins function as regulators of angiogenesis. J Cell Commun Signal *3*, 189-200.

Burwick, N. R., Wahl, M. L., Fang, J., Zhong, Z., Moser, T. L., Li, B., Capaldi, R. A., Kenan, D. J., and Pizzo, S. V. (2005). An Inhibitor of the F1 subunit of ATP synthase (IF1) modulates the activity of angiostatin on the endothelial cell surface. J Biol Chem *280*, 1740-1745.

Cano Mdel, V., Karagiannis, E. D., Soliman, M., Bakir, B., Zhuang, W., Popel, A. S., and Gehlbach, P. L. (2009). A peptide derived from type 1 thrombospondin repeat-containing protein WISP-1 inhibits corneal and choroidal neovascularization. Invest Ophthalmol Vis Sci *50*, 3840-3845.

Carmeliet, P., and Jain, R. K. (2000). Angiogenesis in cancer and other diseases. Nature *407*, 249-257.

Chan-Ling, T., Dahlstrom, J. E., Koina, M. E., McColm, J. R., Sterling, R. A., Bean, E. G., Adamson, S., Hughes, S., and Baxter, L. C. (2011). Evidence of hematopoietic differentiation, vasculogenesis and angiogenesis in the formation of human choroidal blood vessels. Exp Eye Res *92*, 361-376.

Chappelow, A. V., and Kaiser, P. K. (2008). Neovascular age-related macular degeneration: potential therapies. Drugs *68*, 1029-1036.

Clapp, C., Thebault, S., Arnold, E., Garcia, C., Rivera, J. C., and de la Escalera, G. M. (2008). Vasoinhibins: novel inhibitors of ocular angiogenesis. Am J Physiol Endocrinol Metab *295*, E772-778.

Colorado, P. C., Torre, A., Kamphaus, G., Maeshima, Y., Hopfer, H., Takahashi, K., Volk, R., Zamborsky, E. D., Herman, S., Sarkar, P. K., Ericksen, M. B., Dhanabal, M., Simons, M., Post, M., Kufe, D. W., Weichselbaum, R. R., Sukhatme, V. P., and Kalluri, R. (2000). Anti-angiogenic cues from vascular basement membrane collagen. Cancer Res *60*, 2520-2526.

D'Angelo, G., Struman, I., Martial, J., and Weiner, R. I. (1995). Activation of mitogen-activated protein kinases by vascular endothelial growth factor and basic fibroblast growth factor in capillary endothelial cells is inhibited by the antiangiogenic factor 16-kDa N-terminal fragment of prolactin. Proc Natl Acad Sci U S A *92*, 6374-6378.

Dawson, D. W., Pearce, S. F., Zhong, R., Silverstein, R. L., Frazier, W. A., and Bouck, N. P. (1997). CD36 mediates the In vitro inhibitory effects of thrombospondin-1 on endothelial cells. J Cell Biol *138*, 707-717.

Dejana, E. (2010). The role of wnt signaling in physiological and pathological angiogenesis. Circ Res *107*, 943-952.

Do, D. V. (2009). Antiangiogenic approaches to age-related macular degeneration in the future. Ophthalmology *116*, S24-26.

Dvorak, H. F., Brown, L. F., Detmar, M., and Dvorak, A. M. (1995). Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol *146*, 1029-1039.

Egeblad, M., and Werb, Z. (2002). New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer *2*, 161-174.

Faye, C., Moreau, C., Chautard, E., Jetne, R., Fukai, N., Ruggiero, F., Humphries, M. J., Olsen, B. R., and Ricard-Blum, S. (2009). Molecular interplay between endostatin, integrins, and heparan sulfate. J Biol Chem *284*, 22029-22040.

Felbor, U., Dreier, L., Bryant, R. A., Ploegh, H. L., Olsen, B. R., and Mothes, W. (2000). Secreted cathepsin L generates endostatin from collagen XVIII. EMBO J *19*, 1187-1194.

Fukai, N., Eklund, L., Marneros, A. G., Oh, S. P., Keene, D. R., Tamarkin, L., Niemela, M., Ilves, M., Li, E., Pihlajaniemi, T., and Olsen, B. R. (2002). Lack of collagen XVIII/endostatin results in eye abnormalities. EMBO J *21*, 1535-1544.

Gallemore, P. R., and Boyer, D. S. (2006). PDT or Anti-VEGF for AMD treatment. Review of Ophthalmology online.

Gehlbach, P., Demetriades, A. M., Yamamoto, S., Deering, T., Duh, E. J., Yang, H. S., Cingolani, C., Lai, H., Wei, L., and Campochiaro, P. A. (2003). Periocular injection of an adenoviral vector encoding pigment epithelium-derived factor inhibits choroidal neovascularization. Gene Ther *10*, 637-646.

Gonzalez, C., Corbacho, A. M., Eiserich, J. P., Garcia, C., Lopez-Barrera, F., Morales-Tlalpan, V., Barajas-Espinosa, A., Diaz-Munoz, M., Rubio, R., Lin, S. H., Martinez de la Escalera, G., and Clapp, C. (2004). 16K-prolactin inhibits activation of endothelial nitric oxide synthase, intracellular calcium mobilization, and endothelium-dependent vasorelaxation. Endocrinology *145*, 5714-5722.

Green, W. R. (1999). Histopathology of age-related macular degeneration. Mol Vis *5*, 27.

Green, W. R., and Enger, C. (1993). Age-related macular degeneration histopathologic studies. The 1992 Lorenz E. Zimmerman Lecture. Ophthalmology *100*, 1519-1535.

Grossniklaus, H. E., Ling, J. X., Wallace, T. M., Dithmar, S., Lawson, D. H., Cohen, C., Elner, V. M., Elner, S. G., and Sternberg, P., Jr. (2002). Macrophage and retinal pigment epithelium expression of angiogenic cytokines in choroidal neovascularization. Mol Vis *8*, 119-126.

Gunda, V., Wang, S., Sheibani, N., and Sudhakar, A. (2011). Inhibitory effect of tumstatin on corneal neovascularization both in-vitro and in-vivo. J Clinic Experment Ophthalmol (publication ahead of time).

Hamano, Y., Zeisberg, M., Sugimoto, H., Lively, J. C., Maeshima, Y., Yang, C., Hynes, R. O., Werb, Z., Sudhakar, A., and Kalluri, R. (2003). Physiological levels of tumstatin, a fragment of collagen IV alpha3 chain, are generated by MMP-9 proteolysis and suppress angiogenesis via alphaV beta3 integrin. Cancer Cell *3*, 589-601.

Hayashi, M., Tamura, Y., Dohmae, N., Kojima, S., and Shimonaka, M. (2008). Plasminogen N-terminal activation peptide modulates the activity of angiostatin-related peptides on endothelial cell proliferation and migration. Biochem Biophys Res Commun *369*, 635-640.

Hendricks, R. L. (2006). Interaction of angiogenic and immune mechanisms in the eye. Semin Ophthalmol *21*, 37-40.

Ho, T. C., Chen, S. L., Yang, Y. C., Liao, C. L., Cheng, H. C., and Tsao, Y. P. (2007). PEDF induces p53-mediated apoptosis through PPAR gamma signaling in human umbilical vein endothelial cells. Cardiovasc Res *76*, 213-223.

Jager, R. D., Mieler, W. F., and Miller, J. W. (2008). Age-related macular degeneration. N Engl J Med *358*, 2606-2617.

Kaur, S., Kuznetsova, S. A., Pendrak, M. L., Sipes, J. M., Romeo, M. J., Li, Z., Zhang, L., and Roberts, D. D. (2011). Heparan sulfate modification of the transmembrane receptor CD47 is necessary for inhibition of T cell receptor signaling by thrombospondin-1. J Biol Chem *286*, 14991-15002.

Kim, Y. M., Hwang, S., Pyun, B. J., Kim, T. Y., Lee, S. T., Gho, Y. S., and Kwon, Y. G. (2002). Endostatin blocks vascular endothelial growth factor-mediated signaling via direct interaction with KDR/Flk-1. J Biol Chem *277*, 27872-27879.

Lai, C. C., Wu, W. C., Chen, S. L., Xiao, X., Tsai, T. C., Huan, S. J., Chen, T. L., Tsai, R. J., and Tsao, Y. P. (2001). Suppression of choroidal neovascularization by adeno-associated virus vector expressing angiostatin. Invest Ophthalmol Vis Sci *42*, 2401-2407.

Lawler, J. (2000). The functions of thrombospondin-1 and-2. Curr Opin Cell Biol *12*, 634-640.

Lima, E. S. R., Kachi, S., Akiyama, H., Shen, J., Aslam, S., Yuan Gong, Y., Khu, N. H., Hatara, M. C., Boutaud, A., Peterson, R., and Campochiaro, P. A. (2006). Recombinant non-collagenous domain of alpha2(IV) collagen causes involution of choroidal neovascularization by inducing apoptosis. J Cell Physiol *208*, 161-166.

Lu, M., and Adamis, A. P. (2006). Molecular biology of choroidal neovascularization. Ophthalmol Clin North Am *19*, 323-334.

Maeshima, Y., Colorado, P. C., Torre, A., Holthaus, K. A., Grunkemeyer, J. A., Ericksen, M. B., Hopfer, H., Xiao, Y., Stillman, I. E., and Kalluri, R. (2000). Distinct antitumor properties of a type IV collagen domain derived from basement membrane. J Biol Chem *275*, 21340-21348.

Maeshima, Y., Sudhakar, A., Lively, J. C., Ueki, K., Kharbanda, S., Kahn, C. R., Sonenberg, N., Hynes, R. O., and Kalluri, R. (2002). Tumstatin, an endothelial cell-specific inhibitor of protein synthesis. Science *295*, 140-143.

Magnon, C., Galaup, A., Mullan, B., Rouffiac, V., Bouquet, C., Bidart, J. M., Griscelli, F., Opolon, P., and Perricaudet, M. (2005). Canstatin acts on endothelial and tumor cells via mitochondrial damage initiated through interaction with alphavbeta3 and alphavbeta5 integrins. Cancer Res *65*, 4353-4361.

Magnon, C., Opolon, P., Ricard, M., Connault, E., Ardouin, P., Galaup, A., Metivier, D., Bidart, J. M., Germain, S., Perricaudet, M., and Schlumberger, M. (2007). Radiation and inhibition of angiogenesis by canstatin synergize to induce HIF-1alpha-mediated tumor apoptotic switch. J Clin Invest *117*, 1844-1855.

Marneros, A. G., She, H., Zambarakji, H., Hashizume, H., Connolly, E. J., Kim, I., Gragoudas, E. S., Miller, J. W., and Olsen, B. R. (2007). Endogenous endostatin inhibits choroidal neovascularization. FASEB J *21*, 3809-3818.

Marshall, J. (1987). The ageing retina: physiology or pathology. Eye (Lond) *1 (Pt 2)*, 282-295.

Martini, J. F., Piot, C., Humeau, L. M., Struman, I., Martial, J. A., and Weiner, R. I. (2000). The antiangiogenic factor 16K PRL induces programmed cell death in endothelial cells by caspase activation. Mol Endocrinol *14*, 1536-1549.

Mettouchi, A., and Meneguzzi, G. (2006). Distinct roles of beta1 integrins during angiogenesis. Eur J Cell Biol *85*, 243-247.

Miyajima-Uchida, H., Hayashi, H., Beppu, R., Kuroki, M., Fukami, M., Arakawa, F., Tomita, Y., and Oshima, K. (2000). Production and accumulation of thrombospondin-1 in human retinal pigment epithelial cells. Invest Ophthalmol Vis Sci *41*, 561-567.

Monboisse, J. C., Garnotel, R., Bellon, G., Ohno, N., Perreau, C., Borel, J. P., and Kefalides, N. A. (1994). The alpha 3 chain of type IV collagen prevents activation of human polymorphonuclear leukocytes. J Biol Chem *269*, 25475-25482.

Mori, K., Ando, A., Gehlbach, P., Nesbitt, D., Takahashi, K., Goldsteen, D., Penn, M., Chen, C. T., Melia, M., Phipps, S., Moffat, D., Brazzell, K., Liau, G., Dixon, K. H., and Campochiaro, P. A. (2001). Inhibition of choroidal neovascularization by intravenous injection of adenoviral vectors expressing secretable endostatin. Am J Pathol *159*, 313-320.

Nozaki, M., Raisler, B. J., Sakurai, E., Sarma, J. V., Barnum, S. R., Lambris, J. D., Chen, Y., Zhang, K., Ambati, B. K., Baffi, J. Z., and Ambati, J. (2006). Drusen complement components C3a and C5a promote choroidal neovascularization. Proc Natl Acad Sci U S A *103*, 2328-2333.

Nyberg, P., Xie, L., and Kalluri, R. (2005). Endogenous inhibitors of angiogenesis. Cancer Res *65*, 3967-3979.

Oklu, R., Walker, T. G., Wicky, S., and Hesketh, R. (2010). Angiogenesis and current antiangiogenic strategies for the treatment of cancer. J Vasc Interv Radiol *21*, 1791-1805; quiz 1806.

Panka, D. J., and Mier, J. W. (2003). Canstatin inhibits Akt activation and induces Fas-dependent apoptosis in endothelial cells. J Biol Chem *278*, 37632-37636.

Penn, J. S., Madan, A., Caldwell, R. B., Bartoli, M., Caldwell, R. W., and Hartnett, M. E. (2008). Vascular endothelial growth factor in eye disease. Prog Retin Eye Res *27*, 331-371.

Petitclerc, E., Boutaud, A., Prestayko, A., Xu, J., Sado, Y., Ninomiya, Y., Sarras, M. P., Jr., Hudson, B. G., and Brooks, P. C. (2000). New Functions for Non-collagenous Domains of Human Collagen Type IV. Novel integrin ligands inhibiting angiogenesis and tumor growth in vivo. J Biol Chem *275*, 8051-8061.

Rehn, M., Veikkola, T., Kukk-Valdre, E., Nakamura, H., Ilmonen, M., Lombardo, C., Pihlajaniemi, T., Alitalo, K., and Vuori, K. (2001). Interaction of endostatin with integrins implicated in angiogenesis. Proc Natl Acad Sci U S A *98*, 1024-1029.

Ricard-Blum, S., Feraud, O., Lortat-Jacob, H., Rencurosi, A., Fukai, N., Dkhissi, F., Vittet, D., Imberty, A., Olsen, B. R., and van der Rest, M. (2004). Characterization of endostatin binding to heparin and heparan sulfate by surface plasmon resonance and molecular modeling: role of divalent cations. J Biol Chem *279*, 2927-2936.

Roberts, W. G., and Palade, G. E. (1995). Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. J Cell Sci *108 (Pt 6)*, 2369-2379.

Roth, J. M., Akalu, A., Zelmanovich, A., Policarpio, D., Ng, B., MacDonald, S., Formenti, S., Liebes, L., and Brooks, P. C. (2005). Recombinant alpha2(IV)NC1 domain inhibits tumor cell-extracellular matrix interactions, induces cellular senescence, and inhibits tumor growth in vivo. Am J Pathol *166*, 901-911.

Spaide, R. F., Armstrong, D., and Browne, R. (2003). Continuing medical education review: choroidal neovascularization in age-related macular degeneration--what is the cause? Retina *23*, 595-614.

Sudhakar, A., and Boosani, C. S. (2008). Inhibition of tumor angiogenesis by tumstatin: insights into signaling mechanisms and implications in cancer regression. Pharm Res *25*, 2731-2739.

Sudhakar, A., and Kalluri, R. (2010). Molecular mechanisms of angiostatis. Encyclopedia of the eye *3 M-P*, 52-59.

Sudhakar, A., Nyberg, P., Keshamouni, V. G., Mannam, A. P., Li, J., Sugimoto, H., Cosgrove, D., and Kalluri, R. (2005). Human alpha1 type IV collagen NC1 domain exhibits distinct antiangiogenic activity mediated by alpha1beta1 integrin. J Clin Invest *115*, 2801-2810.

Sudhakar, A., Sugimoto, H., Yang, C., Lively, J., Zeisberg, M., and Kalluri, R. (2003). Human tumstatin and human endostatin exhibit distinct antiangiogenic activities mediated by alpha vbeta 3 and alpha 5beta 1 integrins. Proc Natl Acad Sci U S A *100*, 4766-4771.

Tabruyn, S. P., Sorlet, C. M., Rentier-Delrue, F., Bours, V., Weiner, R. I., Martial, J. A., and Struman, I. (2003). The antiangiogenic factor 16K human prolactin induces caspase-dependent apoptosis by a mechanism that requires activation of nuclear factor-kappaB. Mol Endocrinol *17*, 1815-1823.

Takahashi, K., Saishin, Y., Silva, R. L., Oshima, Y., Oshima, S., Melia, M., Paszkiet, B., Zerby, D., Kadan, M. J., Liau, G., Kaleko, M., Connelly, S., Luo, T., and Campochiaro, P. A. (2003). Intraocular expression of endostatin reduces VEGF-induced retinal vascular permeability, neovascularization, and retinal detachment. FASEB J *17*, 896-898.

Tarui, T., Miles, L. A., and Takada, Y. (2001). Specific interaction of angiostatin with integrin alpha(v)beta(3) in endothelial cells. J Biol Chem *276*, 39562-39568.

Tombran-Tink, J., Chader, G. G., and Johnson, L. V. (1991). PEDF: a pigment epithelium-derived factor with potent neuronal differentiative activity. Exp Eye Res *53*, 411-414.

Wang, Y., Yin, H., Chen, P., and Xie, L. (2011). Inhibitory Effect of Canstatin in Alkali Burn-Induced Corneal Neovascularization. Ophthalmic Res *46*, 66-72.

Wickstrom, S. A., Alitalo, K., and Keski-Oja, J. (2002). Endostatin associates with integrin alpha5beta1 and caveolin-1, and activates Src via a tyrosyl phosphatase-dependent pathway in human endothelial cells. Cancer Res *62*, 5580-5589.

Wickstrom, S. A., Alitalo, K., and Keski-Oja, J. (2003). Endostatin associates with lipid rafts and induces reorganization of the actin cytoskeleton via down-regulation of RhoA activity. J Biol Chem *278*, 37895-37901.

Young, R. W., and Bok, D. (1969). Participation of the retinal pigment epithelium in the rod outer segment renewal process. J Cell Biol *42*, 392-403.

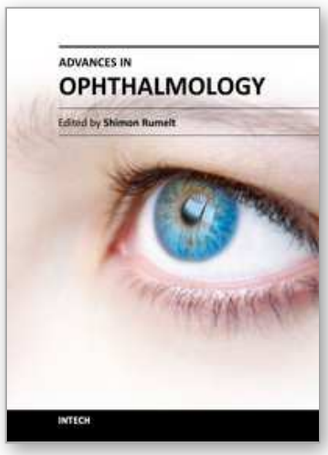
Zerlin, M., Julius, M. A., and Kitajewski, J. (2008). Wnt/Frizzled signaling in angiogenesis. Angiogenesis *11*, 63-69.

Zhang, S. X., and Ma, J. X. (2007). Ocular neovascularization: Implication of endogenous angiogenic inhibitors and potential therapy. Prog Retin Eye Res *26*, 1-37.

Zhou, S. Y., Xie, Z. L., Xiao, O., Yang, X. R., Heng, B. C., and Sato, Y. (2010). Inhibition of mouse alkali burn induced-corneal neovascularization by recombinant adenovirus encoding human vasohibin-1. Mol Vis *16*, 1389-1398.

Zhou, X. Y., Liao, Q., Pu, Y. M., Tang, Y. Q., Gong, X., Li, J., Xu, Y., and Wang, Z. G. (2009). Ultrasound-mediated microbubble delivery of pigment epithelium-derived factor gene into retina inhibits choroidal neovascularization. Chin Med J (Engl) *122*, 2711-2717.

Ziche, M., and Morbidelli, L. (2000). Nitric oxide and angiogenesis. J Neurooncol *50*, 139-148.



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