

VISION ACADEMY LITERATURE SUMMARY

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VEGFR1 Signaling in Retinal Angiogenesis and Microinflammation

Background

Intravitreal anti-vascular endothelial growth factor (anti-VEGF) therapy can provide fast and sustained improvements in visual acuity (VA) and is considered the gold standard treatment for retinal diseases including age-related macular degeneration (AMD) and diabetic retinopathy (DR).¹ Anti-VEGF treatments target VEGF ligands,¹ preventing them from binding to and activating VEGF receptors, and the varying molecular features of these drugs contribute to their unique safety and efficacy profiles.²

Three VEGF receptors (VEGFR1, VEGFR2, and VEGFR3) and seven VEGF ligands (VEGF-A, -B, -C, -D, -E, -F, and placental growth factor [PlGF]) are known to exist in humans and each has different functions and ligand-binding properties.^{2,3} For example, VEGF-A binds to and activates the VEGF receptors VEGFR1 and VEGFR2, while PlGF and VEGF-B only bind to and activate VEGFR1.²

Although both VEGFR1 and VEGFR2 are implicated in retinal disease, research has mostly focused on VEGFR2,² which has been shown to induce angiogenesis and increase vascular permeability when activated by VEGF-A in endothelial cells.³ Meanwhile, the biology of VEGFR1 remains more elusive, in part because the ligand-receptor interactions are more complex for VEGFR1. However, it has been well established that VEGFR1 acts, at least in part, as a “decoy” receptor by competitively binding VEGF-A and attenuating its signaling via VEGFR2.⁴

Research has also shown the importance of direct VEGFR1 signaling, specifically in relation to angiogenesis, vascular permeability, and microinflammatory cascades within the retina. VEGFR1 activation by any of its ligands can affect vascular permeability and induce macrophage and microglia production of pro-inflammatory and pro-angiogenic mediators.² As VEGF-A, VEGF-B, and PlGF all bind and activate VEGFR1, competition exists for the receptor's binding site, which can impact VEGFR2 signaling as it can be indirectly activated by ligands for which it has no affinity. This occurs when the binding of these ligands to VEGFR1 result in an increased proportion of free VEGF-A for which VEGFR2 does have an affinity.² Meanwhile, direct activation of VEGFR1 signaling by PlGF has been shown to result in the stimulation of endothelial growth in ischemic retina, as well as the transmission of angiogenic signals.⁵ Furthermore, although VEGF and PlGF both bind VEGFR1, it is thought that each activates the receptor in a unique manner,⁶ but might also have the ability to interact to propagate an increased response.⁷

This literature summary focuses on the publication by Uemura et al.,² which explores the current evidence on the role of VEGFR1 in retinal diseases.

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Signaling via VEGFR1

Expression of VEGFR1 has been detected in a variety of retinal cell types, including retinal and choroidal endothelial cells, pericytes, retinal and choroidal mononuclear phagocytes (MPs), Müller cells, photoreceptor cells, and retinal pigment epithelial cells (**Figure 1A**).² Activation of VEGFR1 by VEGF-A or PlGF alone or as a heterodimer induces signaling cascades through multiple pathways, leading to gene transcription and the promotion of pathological processes involved in angiogenesis in endothelial cells and pericytes in the choroid and retina. These include pericyte ablation, loss of tight junctions between endothelial cells, vasodilation, blood–retina barrier (BRB) breakdown, increased vascular permeability, edema and hemorrhage in the surrounding tissue, macrophage migration, increased angiogenic sprouts,

and neoangiogenesis (**Figure 1B**).² The consequences of excess VEGFR1 signaling in the choroid and retina differ between cell types. In retinal pigment epithelial cells, excess signaling causes neoangiogenesis of vessels through Bruch's membrane into the retinal pigment epithelium and loss of retinal pigment epithelial cells. In photoreceptor cells, it results in rod death, cone segment loss, and a reduction in photoreceptor integrity, while in Müller cells, it causes Müller cell activation. In microglial cells, excess signaling leads to the recruitment, accumulation, and activation of microglial cells and other retinal macrophages, which results in the release of pro-inflammatory cytokines and the subsequent development of hyper-reflective foci (**Figure 1C**).²

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The Vision Academy is sponsored by Bayer. This document was prepared on behalf of the Vision Academy by Akiyoshi Uemura, Marcus Fruttiger, Patricia A. D'Amore, Sandro De Falco, Antonia M. Jousseaume, Florian Sennlaub, Lynne R. Brunck, Kristian T. Johnson, George N. Lambrou, Kay D. Rittenhouse, and Thomas Langmann.

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Figure 1. VEGFR1 and its signaling in the choroid and retina.

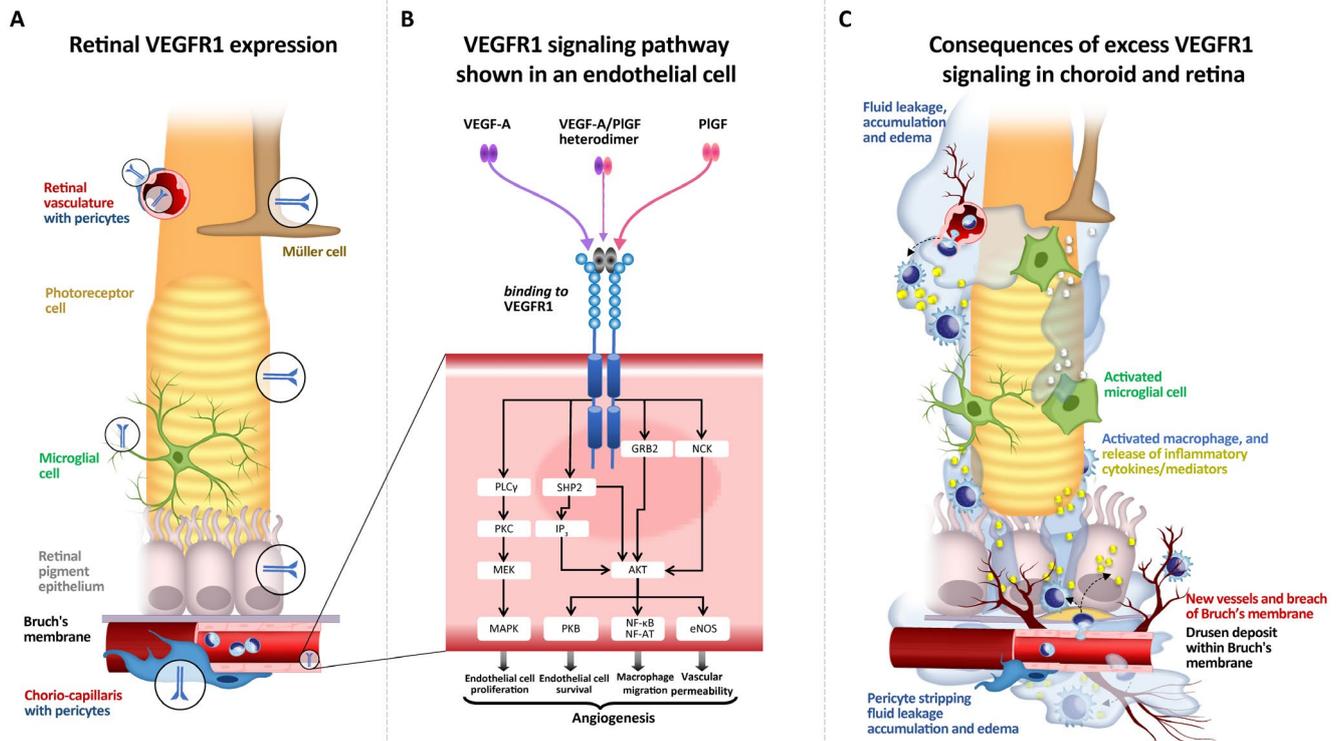


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Biological functions of VEGFR1

1. Angiogenesis

Loss of vision in retinal disease is due to pathologic retinal angiogenesis.⁸ VEGFR1 and VEGFR2 are expressed on the cell surface of most blood endothelial cells⁹ and retinal angiogenesis depends on both the gradient of VEGF-A and its concentration.¹⁰ Therefore, agonistic activity of VEGF-A on VEGFR2 can be balanced by sequestration of the ligand by VEGFR1.² The direct role of VEGFR1 signaling in angiogenesis has not been fully characterized and although higher levels of VEGFR1 ligands can increase angiogenesis, results are often context-dependent due to the complex nature of VEGFR1 signaling.²

2. Vascular permeability

The inner retina is dependent on the retinal vasculature and inner BRB, while the outer retina is maintained by the highly permeable choroidal vasculature and outer BRB.² Although largely attributed to the actions of VEGF-A/VEGFR2, vascular permeability can be controlled by VEGFR1 via activation of endothelial nitric oxide synthase,² as stimulated by VEGF-A.¹¹

3. Inflammation

VEGFR1 is expressed by MPs such as microglia and macrophages,⁷ which play important immunological roles in the retina. Chronic microinflammation can be associated with MPs² and can contribute to various retinal pathologies.¹² In retinal disease, VEGFR1 activation causes macrophages and microglia to produce pro-inflammatory and pro-angiogenic mediators (Figure 1C), including certain cytokines and VEGF-A.² Although the ways in which VEGF ligands affect the VEGFR1 signal in macrophages have yet to be elucidated, it is thought that macrophage-derived VEGF and/or PlGF activate VEGFR1.¹³

The role of VEGFR1 in retinal diseases

I. Diabetic retinopathy and diabetic macular edema

Diabetes can impact the retinal neovascular unit and its vascular, neuronal, glial, and immune cells.¹⁴ Levels of PlGF are elevated in aqueous humor samples taken from patients with diabetic macular edema (DME) and proliferative DR (PDR),¹⁵ while levels of PlGF and VEGF-A increase with levels of ischemia (i.e., increases from the diabetic state to PDR, and to neovascular glaucoma).¹⁶ In a pericyte-deficient retina, VEGFR1 facilitates motility of MPs (Figure 1B) which overexpress both VEGF-A and PlGF.¹³ Overexpression of PlGF has been shown to cause microaneurysms and vascular sprouts in the retinal vasculature of rats, as well as glial activation and proliferation¹⁷ which are linked to DR pathology. In addition, the absence of PlGF prevents retinal cell death, capillary degeneration, pericyte loss, and BRB breakdown, highlighting the involvement of PlGF and VEGFR1 in DR.² Anti-VEGF therapies have shown differences in efficacy and durability in DME studies,² which may be a result of differences in relative VEGF-A potency or binding affinity between these agents, or may reflect specificity for VEGF-A only versus blockade of VEGFR1 via inhibition of all three ligands (VEGF-A, PlGF, and VEGF-B).¹⁸

II. Retinal vein occlusion

Branch retinal vein occlusion (BRVO) and central retinal vein occlusion (CRVO) can lead to retinal ischemia,² which can cause inflammation and associated vascular remodeling.¹⁹ Macular edema is an additional outcome of these angiogenic and inflammatory processes, which are mediated by VEGF.¹⁹ In patients with CRVO or BRVO with macular edema, levels of soluble VEGFR1 (sVEGFR1) were positively correlated with PlGF levels and inflammatory factors,^{19,20} which may implicate the VEGFR1-mediated activation of microglia and macrophages.²

Anti-VEGF therapies effectively improve VA and reduce macular edema in patients with RVO and have shown good efficacy regardless of whether they target VEGF-A only or both VEGF-A and PlGF.² A study in patients with RVO has, however, demonstrated the need for less frequent treatment²¹ when using anti-VEGF agents that target multiple ligands.²² This is similar to the observation in patients with DME, where patients with worse VA at baseline experienced greater gains in VA when treated with anti-VEGF agents targeting multiple ligands.^{23,24}

III. Age-related macular degeneration

A key characteristic of the neovascular form of AMD is unregulated angiogenesis from the subretinal space into the retina, resulting in the formation of leaky vessels.²⁵ Laser choroidal neovascularization (CNV) murine models, which are used to mimic the features of exudative AMD, display rapid recruitment of MPs and mechanisms of CNV occurring within a few days,²⁵ and the development of CNV can be inhibited by blockade of VEGFR1.²⁶ A growing body of evidence suggests that expression of VEGFR1 is greater than that of VEGFR2 in monocytes and other cells associated with AMD.² Additionally, PlGF inhibition has been found to decrease the volume of activated subretinal MPs and reduce vessel leakage associated with CNV.⁷ This effect was higher when blocking both VEGF-A and PlGF compared to PlGF alone, suggesting an interaction of VEGF-A and PlGF via VEGFR1.⁷ It is therefore likely that VEGFR1 signaling in monocyte recruitment and activation of MPs are both mediated by VEGF-A and PlGF, thus contributing to the retinal inflammation seen in AMD.² It is expected that any anti-VEGF therapy inhibiting VEGFR1 might control this pathogenic inflammation.

The role of VEGFR1 ligands

The functional roles of the VEGFR1 ligands in various retinal pathologies are far from clear, but this summary highlights that both PIGF and VEGF contribute to pathologic processes in retinal disease. Importantly, this could mean that therapeutic interventions targeting a single ligand will not be as effective as those that target all VEGFR1 ligands (which has been seen in some studies).^{2,7,18}

Both preclinical and clinical studies have examined potential mechanisms. Studies in animal models have highlighted the importance of PIGF and VEGFR1: as well as the aforementioned overexpression of PIGF-causing processes linked to DR pathology,¹⁷ PIGF deficiency prevented diabetes-induced retinal cell death, capillary degeneration, and vascular leakage in diabetic mouse strains.²⁷ Additionally, anti-VEGFR1 antibodies were more effective at preventing BRB breakdown than those specific to VEGFR2, indicating that directly blocking VEGFR1 signaling can affect vascular permeability or inflammation in retinopathy of prematurity.²⁸ Clinical studies have also provided insights into the importance of VEGFR1 and its

ligands. As previously discussed, PIGF levels were elevated in patients with CRVO, but this study also demonstrated that levels of ligands and inflammatory cytokines were correlated with aqueous levels of sVEGFR1 and not sVEGFR2.¹⁹ This suggests an anti-VEGFR1 therapy might be more effective for treating macular edema. Furthermore, increased levels of sVEGFR1 and systemic inflammation during the first postnatal month have been shown to increase the risk of retinopathy of prematurity.²⁹

Preclinical and clinical insights such as these are collectively shaping the understanding behind why some trials have shown outcome differences between drugs that target single versus multiple ligands. The results of comparative trials could provide insights regarding the benefit of specific anti-VEGF agents with different target profiles. To understand the differentiating effects of blocking PIGF versus VEGF-A or VEGF-B, there is a need to clarify a role for VEGFR1/PIGF in the pathobiology of choroidal vascular diseases.

Further considerations

Elucidating the role of VEGFR1 in the context of retinal disease is complicated by the complexities of its biological pathways, as well as the action of its ligands and interactions with VEGFR2. It is therefore important to study the full range of relevant molecular interactions between VEGF-A, VEGF-B, and PIGF. As research continues, our understanding of the contributions of these ligands through VEGFR1 to disease mechanisms and the interplay between microinflammation and angiogenesis in these diseases should improve. Further research into processes underlying the pathology of retinal disorders may therefore offer insight into the mechanistic basis behind variations in the efficacy of VEGF inhibitors.

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