

# Vascular Endothelial Growth Factor: from discovery to therapy

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## Abstract

Blood vessels are fundamental to life, facilitating delivery of vital nutrients to tissues. If vascular function is interrupted tissues may perish, as occurs in many disease states. Understanding basic vascular biology is essential for the identification of potential therapeutic targets and the development of treatment modalities. A key success story in the translation of basic science research to clinical medicine is that of vascular endothelial growth factor (VEGF). It was identified in relation to increased vascularity associated with tumour growth, and work began to define its role in human health and disease. The role of VEGF in ocular neovascular disease became particularly apparent in the pathogenesis of age-related macular degeneration (AMD), directly leading to the development of anti-VEGF therapy. VEGF is also central to normal retinal vascular development, and when interrupted by premature birth, retinopathy of prematurity can result. The successful translation of VEGF research into effective therapy has heralded much success in ophthalmology practice.

**Keywords:** VEGF, vascular endothelial growth factor, age-related macular degeneration, retinopathy

## Introduction

Blood vessels are the conduits of life. The complex process of angiogenesis, resulting in established microvascular networks, is fundamental for pre- and post-natal development and adult tissue repair. However, despite laying foundations for life, angiogenesis can also be deadly. When dysregulated, the growth of new blood vessels can contribute to pathogenesis of many malignant, inflammatory, ischaemic, infectious and immune disorders. Ocular angiogenesis underlies neovascular disorders of the cornea, retina and choroid, and can cause irreversible visual impairment due to corneal opacification or deleterious modification of retinal neuronal architecture.

Angiogenesis results from the complex interaction of multiple growth factors, extracellular matrix molecules, vascular endothelial cells and cell signalling molecules. Understanding this process has been the focus of decades of research, providing insight into many life- and sight-threatening diseases. This understanding has allowed identification of therapeutic opportunities that have translated into several clinical success stories. With an estimated 500 million people worldwide that

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could benefit from pro- or anti-angiogenic therapy, angiogenesis research is likely to revolutionise medicine in future decades.<sup>1</sup>

The story of vascular endothelial growth factor (VEGF) is a key example of this successful translation of basic vascular biology to clinical medicine, particularly ophthalmic medicine. This report will follow the story of VEGF from initial discovery, define its role in adult and developmental ocular vascular biology, and explore its pathogenic role in ocular disease as well as reviewing the development of anti-VEGF treatment modalities and their efficacy in two common blinding diseases.

## Identification of VEGF

VEGF research unknowingly began over a century ago with Rudolf Virchow's observation that increased vascularity often accompanies tumour growth.<sup>2</sup> In 1939, a "blood vessel growth-stimulating factor" was postulated to derive from tumours,<sup>3</sup> and subsequently, diffusible molecules were shown to mediate tumour angiogenesis.<sup>4</sup> These observations led to the proposition of anti-angiogenesis as a potential anti-cancer strategy and fuelled a the race to identify regulators of blood vessel growth.

Two independent lines of research resulted in VEGF identification. In 1983, Senger et al. identified in tumour cells a protein that induced vessel leakage, which they named vascular permeability factor (VPF).<sup>5</sup> Human VPF was purified and sequenced several years later.<sup>6</sup> A parallel line of research highlighted the degree of cell mitosis and division when bovine pituitary follicular cell-conditioned medium was cultured with endothelial cells.<sup>7</sup> A secreted molecule was believed responsible, which was subsequently isolated, purified and the amino-acid sequence identified. This protein, once proven novel, was named 'vascular endothelial growth factor' (VEGF).<sup>7</sup> Later screening of human DNA libraries revealed complimentary DNA clones, encoding multiple molecular species of VEGF, most likely due to alternative mRNA splicing.<sup>8</sup> Surprisingly, one VEGF variant was identical to recently identified human VPF,

suggesting that a single molecule had both permeability-enhancing and mitogenic activity.

Whether VEGF functions as an angiogenic factor *in vivo* was the next question to be answered. Low VEGF expression was detected in avascular ovary granulosa cells, whereas in the highly vascular corpus luteum VEGF was found to be up-regulated.<sup>9</sup> VEGF is also strongly up-regulated in the highly vascular glioblastoma multiforme,<sup>10</sup> providing further evidence of VEGF expression and blood vessel growth correlation. This supported the fact that *in vivo* endothelial cells do selectively express high-affinity VEGF binding sites<sup>11</sup> makes VEGF an attractive candidate for an angiogenic regulator. Having been identified, evidence of VEGF activity in many organ systems, such as the eye, began to emerge.

## VEGF and the adult eye

In a similar period, related ophthalmic research had also detected the presence of VEGF. A preliminary report published in 1980 not only demonstrated the angiogenic activity of retinal extracts, but also revealed the angiogenic properties of vitreous from patients with intraocular neovascularization.<sup>12</sup> Once VEGF had been identified, its synthesis and secretion by retinal pigment epithelial (RPE) cells was demonstrated.<sup>13</sup>

The discovery of a retina-derived angiogenic factor, induced by hypoxia, was particularly significant as it represented a promising target for the driving force behind pathological retinal neovascularization. In proliferative retinopathies such as those associated with diabetes and age-related macular degeneration (AMD), neovascularization often follows retinal ischaemia induced by capillary closure. Indeed, following induction of retinal ischaemia by laser occlusion in non-human primates, VEGF levels were found to change proportionately and synchronously with neovascularization severity.<sup>14</sup> Although the liberation of a diffusible angiogenic factor by ischaemic retina, leading to pathological neovascularization, had long been postulated,<sup>15</sup> the identity was not confirmed until high VEGF expression

was detected in the fluid of eyes with ischaemic retinal disorders, such as proliferative diabetic retinopathy.<sup>16</sup> Soon after, VEGF was identified in post-mortem tissues of AMD patients,<sup>17</sup> and its participation in AMD pathogenesis began to unravel.

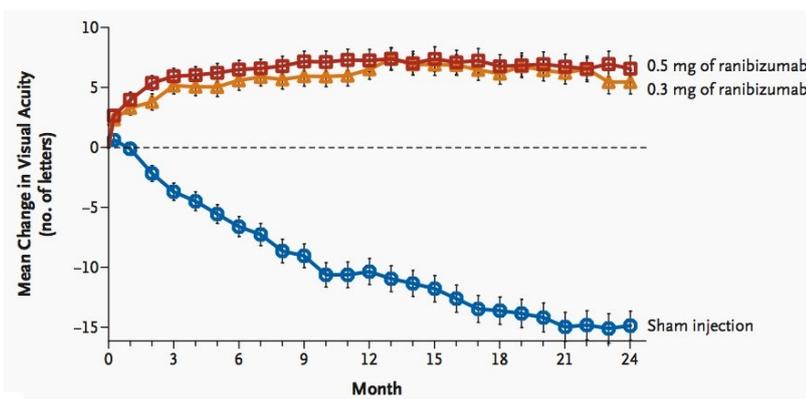
### **VEGF in age-related macular degeneration**

AMD represents the leading cause of severe, irreversible visual impairment in the United Kingdom.<sup>18</sup> It is a complex, multi-stage disorder ranging from sub-clinical changes at the choroid-RPE interface, to advanced non-exudative geographic atrophy, or exudative neovascular disease, both associated with degenerative macular dysfunction. Despite the exudative form only accounting for 10-20% of cases, this form is responsible for up to 90% of visual impairment associated with the disease.<sup>19</sup> It is hallmarked by choroidal neovascularization (CNV), creating a weak neovascular network prone to leakage and haemorrhage, before evolving into fibrovascular tissue. Initial insults to visual function result from retinal oedema and vitreous haemorrhage, but as disease progresses, proliferating vessels penetrate Bruch's membrane and invade the subretinal space, destroying the RPE and photoreceptors in its path.<sup>20</sup> End-stage AMD involves CNV involution and formation of a dense submacular disciform scar. The precise mechanism underlying conversion to exudative AMD is unknown, however VEGF appears to play a central role. Neovascular tissues surgically excised from AMD patients were found to contain VEGF,<sup>17</sup> and due to its well-known angiogenic and permeability-enhancing properties, it was soon regarded as a key mediator of exudative AMD. However, a transgenic model of raised VEGF production demonstrated intrachoroidal neovascularization that failed to penetrate Bruch's membrane, suggesting raised VEGF alone is insufficient to cause the subretinal neovascularization seen in AMD.<sup>21</sup> Therefore additional insult to Bruch's membrane integrity must occur in AMD pathogenesis. The quiescent state of healthy choroidal endothelial cells is attributed to a fine balance between pro- and

anti-angiogenic factors, with CNV resulting from a disruption in the balance, favouring pro-angiogenic signalling.<sup>22</sup> Subsequent studies highlighted such a misbalance, specifically between VEGF and the anti-angiogenic pigment epithelium derived factor.<sup>23</sup> This misbalance could be caused by pathological states, such as ischaemia, inflammation or hypoxia, acting independently or in concert.<sup>20</sup> AMD research points to a multifactorial aetiology, involving both genetic and environmental factors interacting to create a misbalance in growth factors, proteolytic enzymes and inflammatory mediators. Common to exudative AMD however, is the pathological expression of VEGF; and understanding this led to successful treatment of an otherwise invariably blinding disease.

### **Anti-VEGF treatment for age-related macular degeneration**

By identifying VEGF in AMD pathogenesis, scientists had identified a potential therapeutic target. In 1997, a humanised anti-VEGF monoclonal antibody, known as bevacizumab, was developed that potently suppressed angiogenesis and ischaemic retinal neovascularization.<sup>24</sup> Subsequent pre-clinical studies confirmed that intravitreal anti-VEGF agents suppressed CNV,<sup>25</sup> giving rise to theories of its potential for treatment of exudative AMD. Proof of this concept was provided when a clinical study reported improved clinical outcomes in AMD patients receiving intravitreal injections of pegaptanib, a VEGF aptamer, compared with sham injections.<sup>26</sup> Although progression of visual loss was slowed, few patients experienced improved visual acuity. Shortly after, two large phase III clinical trials reported on ranibizumab, an affinity-matured anti-VEGF humanised monoclonal antibody fragment that binds and potently neutralises all VEGF isoforms,<sup>27</sup> and penetrates all retinal layers.<sup>28</sup> The MARINA trial reported a greater avoidance of moderate vision loss in patients receiving ranibizumab compared with sham injections, with patients even gaining visual acuity (Figure 1).<sup>29</sup> Similar results were reported from the ANCHOR trial, in which a greater proportion of ranibizumab-treated



**Figure 1 | Mean changes in visual acuity with ranibizumab and sham injections.**<sup>29</sup> The mean changes from baseline of visual acuity over a 24-month period with 0.3mg ranibizumab, 0.5mg ranibizumab and sham injections. Reproduced with permission from © 2006 Massachusetts Medical Society.

patients avoided moderate visual loss compared with those receiving verteporfin photodynamic therapy.<sup>30</sup> Again, those treated with ranibizumab even gained visual acuity.

The introduction of effective anti-VEGF treatment for exudative AMD marked a conceptual revolution in the therapeutic approach to ocular neovascular disorders. Blocking free VEGF with aptamers or antibodies was just the first step, with new classes of drugs targeting either VEGF and VEGFRs at the nuclear translational level,<sup>31</sup> through kinase inhibitors for interruption of intracellular cascades.<sup>32</sup> These new treatment modalities are expected to provide greater efficacy. What remains unanswered however, is whether greater efficacy will be accompanied by reduced safety and greater side effects, as VEGF is known to play an important role in normal adult tissue homeostasis. Current drugs lack clinically detectable side-effects, and electrophysiological tests even show recovery of photoreceptors at sites of CNV.<sup>33</sup> Whether long-term side-effects will occur still remains unknown.

## VEGF and the developing eye

As knowledge of VEGF's contribution to adult ocular health and disease was expanding, developmental scientists were elucidating its central role in ocular vascular development. In choriocapillari development, VEGF is essential, proven by the failure of choroidal development in VEGF non-expression by retinal pigment epithelial (RPE) cells.<sup>34</sup> Extensive research has similarly shown VEGF to be key in retinal vasculature development.

The human retina is among the last organs to be vascularized.<sup>35</sup> Development of the superficial plexus is initiated as astrocytes, emanating from the optic nerve, migrate towards the periphery.<sup>36</sup> As they spread across the retina they experience hypoxia and express VEGF, thus stimulating the expanding vessel network to grow over them.<sup>37</sup> The astrocytes ensheath the new blood vessels, contributing to blood-retinal barrier formation.<sup>38</sup> Following development of the retinal arcades, new vessels sprout from pre-existing vessels,<sup>39</sup> forming the peripheral, perifoveal and deep retinal vessels, and the capillary system.<sup>40</sup> Endothelial tip cells of new vessels are guided by VEGF gradients; they generate filopodia (cytoplasmic projections) that express VEGFR-2, mediating migration towards high VEGF concentrations.<sup>41</sup> This gradient is generated by vascularized, normoxic retina down-regulating VEGF expression, with hypoxia in areas of non-vascularized retina stimulating VEGF production. When this gradient is artificially flattened, retinal vascular growth is disrupted.<sup>41</sup> Once laid down, the primitive capillary plexus remodels and matures into a hierarchical vascular tree, as vessel diameters change and arteries and veins differentiate.<sup>42</sup> Regression and reduction of vessel density occurs in areas of rich oxygen supply, adjacent to arteries.<sup>39</sup> When hyperoxia is induced, VEGF expression is suppressed and retinal vessels fail to form,<sup>37</sup> highlighting the central role of VEGF in orchestration of retinal vascular development. Defining the normal development of retinal vasculature allowed understanding of disorders, such as retinopathy of

prematurity (ROP), that occur when development is interrupted.

### VEGF and retinopathy of prematurity

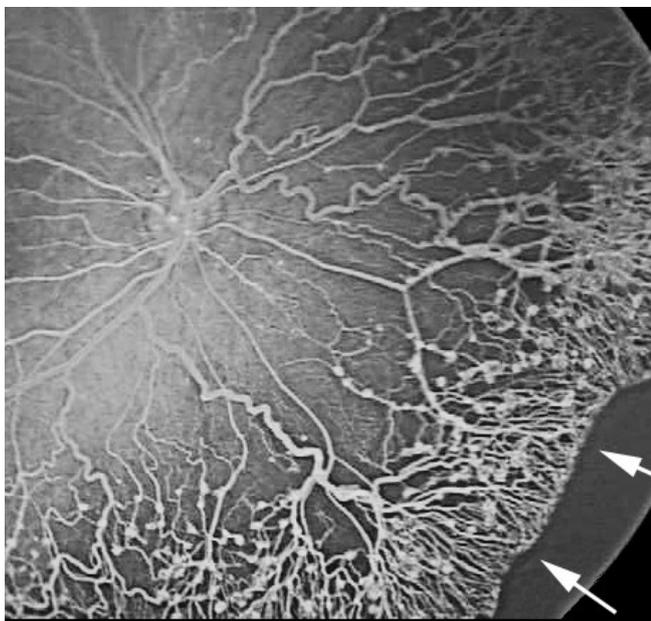
ROP is the leading cause of childhood blindness in developed countries.<sup>43</sup> It is a neovascularizing disease of preterm neonates, the incidence of which strongly correlates with earlier gestational age.<sup>35</sup> ROP results when the physiological conditions supporting normal retinal vascular development are significantly interrupted. Research has highlighted how critical the hypoxic uterine environment is for normal vascular development.<sup>37</sup> If infants are delivered before vascularization is complete, usually at 36-40 weeks gestational age,<sup>40</sup> development must then continue in the hyperoxic extra-uterine environment leading to deranged vessel development (Figure 2).

The developmental abnormality occurs in two phases: a vaso-obliterative phase followed by a vaso-proliferative phase.<sup>44</sup> Birth of the preterm infant initiates the vaso-obliterative phase, as PaO<sub>2</sub> jumps from 30-35mmHg in utero to 55-80mmHg extra

utero.<sup>40</sup> This relative hyperoxia down-regulates VEGF expression, causing cessation of vessel growth and constriction and retraction of pre-formed vessels.<sup>44</sup> This process is exacerbated by supplemental oxygen therapy.<sup>45</sup> Then, as the developing retina becomes metabolically active, the incompletely vascularized areas have unmet metabolic demands. The resulting hypoxia triggers VEGF up-regulation, thus initiating the vaso-proliferative phase in which exuberant vessel formation occurs.<sup>44</sup> As in CNV, the neovascular networks are weak and prone to leakage. They grow into the vitreous and cause vitreous haemorrhage. Fibrous changes occurring after vessel regression cause macular dragging and tractional retinal detachment, contributing to visual loss.<sup>40</sup> Understanding the pivotal role of VEGF in ROP pathogenesis has prompted the experimental use of anti-VEGF therapy in ROP patients, with promising results.

### Anti-VEGF treatment in retinopathy of prematurity

Conventional ROP treatment involves peripheral retinal ablation by laser photocoagulation or cryotherapy, and surgical repair of retinal detachment.<sup>46</sup> These treatments are limited, in efficacy, and ROP patients can suffer long-term effects from the treatment itself. The identification of VEGF in ROP pathogenesis, and success of anti-VEGF therapies in other ocular neovascular diseases, led to a series of case reports in which VEGF inhibitors were used in aggressive cases of ROP.<sup>47, 48</sup> These reports described regression of fibrovascular proliferation, iridal neovascularization and ROP, with no ocular or systemic side-effects, providing promising evidence in support of anti-VEGF therapy. A larger trial investigated the use of bevacizumab in 11 infants with moderate and severe ROP, which reported successful treatment following a single injection, with avoidance of retinal detachment, strong myopia and macular ectopia, and without encountering any ocular or systemic complications.<sup>49</sup> These preliminary results advocate modulation of retinal VEGF as a feasible treatment modality for ROP. A large, randomized,



**Figure 2 | Deranged vascular development in ROP.** Fluorescein angiography visualizes the retinal vasculature in a premature baby suffering from ROP. The vasculature has failed to spread across the entire retina (arrows) and hypoxic peripheral retina induces abnormal vessel growth. Image courtesy of Professor Birgit Lorenz, Dept. of Ophthalmology, Justus-Liebig-University Giessen, UKGM Giessen Campus.

controlled, multi-centre trial of intravitreal bevacizumab monotherapy in 143 infants with stage 3+ ROP recently reported a significant benefit compared with conventional laser photocoagulation, although peripheral retinal vessels continued development.<sup>50</sup> Seeing as laser therapy permanently destroys the peripheral retina, these result herald great promise. Larger trials are however necessary to investigate any adverse effects and to establish a safe treatment dosage. It is crucial that these infants undergo close follow-up for the identification of any long-term ocular or neurological adverse consequences, and for comparison of visual outcome with conventional laser therapy. Certainly based on current research anti-VEGF therapy holds great promise and has the potential to become the mainstay of treatment for ROP.

## Conclusion

Understanding vascular biology has been, and continues to be, the focus of vast research and experimental efforts. The story of VEGF is a key example. The identification of VEGF, followed by description of its functional properties, facilitated the characterisation of its role in pathogenesis of many diseases both ocular and extra-ocular. This understanding led directly to development of treatment modalities based on its inhibition, which continue to benefit thousands of patients suffering from blinding and life-threatening diseases. ■

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### LEARNING POINTS

- Vascular endothelial growth factor (VEGF) was first identified in cancer research, fuelling concerted research efforts to define its role in human health and disease.
- VEGF plays a central role in the pathogenesis of ocular neovascular disorders, such as age-related macular degeneration; knowledge which has led to the development of highly successful anti-VEGF therapy.
- In the developing eye, VEGF is essential for the normal development of retinal vasculature, and when interrupted can lead to retinopathy of prematurity - a further promising target for anti-VEGF therapy.

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