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Perspective

VEGF: From Discovery to Therapy: The Champalimaud Award Lecture

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Purpose: Intraocular vascular diseases are leading causes of adult vision loss, and in the mid-1900s, I. C. Michaelson postulated that the retina releases a soluble, diffusible factor that causes abnormal vascular growth and leakage. What became known as "Factor X" eluded investigators for decades.

Methods: The field of cancer research, where Judah Folkman pioneered the concept of angiogenesis, provided the inspiration for the work honored by the 2014 Champalimaud Vision Award. Recognizing that tumors recruit their own blood supply to achieve critical mass, Dr Folkman proposed that angiogenic factors could be therapeutic targets in cancer. Napoleone Ferrara identified vascular endothelial growth factor (VEGF) as such an angiogenic agent: stimulated by hypoxic tumor tissue, secreted, and able to induce neovascularization. VEGF also was a candidate for Factor X, and the 2014 Champalimaud Laureates and colleagues worked individually and collaboratively to identify the role of VEGF in ocular disease.

Results: The Champalimaud Laureates correlated VEGF with ocular neovascularization in animal models and in patients. Moreover, they showed that VEGF not only was sufficient, but it also was required to induce neovascularization in normal animal eyes, as VEGF inhibition abolished ocular neovascularization in key animal models.

Conclusions: The identification of VEGF as Factor X altered the therapeutic paradigms for age-related macular degeneration (AMD), diabetic retinopathy, retinal vein occlusion, and other retinal disorders.

Translational Relevance: The translation of VEGF from discovery to therapy resulted in the most successful applications of antiangiogenic therapy to date. Annually, over one million patients with eye disease are treated with anti-VEGF agents.

On behalf of the laureates, I want to thank the Champalimaud Foundation; in particular, its President Leonor Beleza, who leads the foundation with such wisdom and grace, and the members of the Champalimaud Vision Award Jury. Of course, I also would like to acknowledge the vision and generosity of its founder, António Champalimaud. The Champalimaud Foundation challenges us to conquer the unknown, much like the Portuguese explorers who set sail from her shores. The Champalimaud Vision Award recognizes contributions in vision research, and in alternate years, it is given to groups delivering care in developing countries. However, the Foundation also supports an internal research program in neuroscience and cancer research, as well as a clinical cancer care in its Centre for the Unknown in Lisbon. It is well worth a visit.

I want to thank the other Champalimaud Laureates for allowing me to represent them. We all are honored by the award, and recognize that the group involved in translating the discovery of vascular endothelial growth factor (VEGF) to therapy is very much larger, and includes many other investigators, a few of whom I will mention. However, there also were many postdoctoral fellows, students, and clinicians, as well as industry scientists, and of course patients, whose important contributions I wish to acknowledge.

In the 1990s, the treatment of many retinal diseases was rather grim. For neovascular age-related macular degeneration (AMD), all we really had was a destructive laser treatment, which cauterized the vessels but also caused destruction of the neurosen-

Figure 1. Researchers involved in the discovery of VEGF as Factor X and translation to therapy. *Top row* (*left* to *right*): Judah Folkman, Harold Dvorak, Napoleone Ferrara, Evangelos Gragoudas, Donald D'Amico, Lloyd Paul Aiello. *Bottom row* (*left* to *right*): George King, Lois Smith, Eric Pierce, Patricia D'Amore, David Shima, Anthony Adamis, and Joan Miller.

sory retina, leading to scotomas, or blind spots, and often decreased vision. This was not very rewarding, either for the patient or the treating clinician. Diabetic retinopathy was better controlled with laser photocoagulation, but even so, there were drawbacks and side effects. Treatment for retinal vein occlusion (RVO) also was of limited benefit.

Early in the 1990s, there was a confluence of researchers, especially in Boston, who were interested in ocular neovascularization (Fig. 1). Together and separately, we investigated models of ocular neovascularization in human disease. We explored findings from tumor angiogenesis in ocular disease. Among these investigators, there was Dr Judah Folkman, who was, indeed, the "Father of Angiogenesis"; and Harold Dvorak, who identified a soluble factor known as vascular permeability factor (VPF);[1] Napoleone Ferrara (Genentech), who isolated VEGF and recognized its role in angiogenesis;[2] and Evangelos Gragoudas and Donald D'Amico (Massachusetts Eye and Ear), who provided great clinical insight and leadership. At Harvard Medical School, there were two primary groups investigating VEGF and ocular angiogenesis. One was led by Lloyd Paul Aiello and George King at Joslin Diabetes Center, who later were joined by Lois Smith and Eric Pierce of Boston Children's Hospital. Another group was led by Patricia D'Amore and her graduate student David Shima, along with Anthony Adamis and me, at Boston Children's Hospital and Massachusetts Eye and Ear. Of course, we had many collaborators, postdoctoral fellows, and students who were instrumental in carrying out this work.

The search for a secreted "Factor X" that stimulated ocular neovascularization dates back at least to 1948, when Michaelson postulated that a soluble and diffusible growth factor was responsible for retinal vascular growth in development and disease.[3] Clinicians recognized that damaged or ischemic retina secreted a factor that could leak out into other parts of the eye and cause new blood vessel

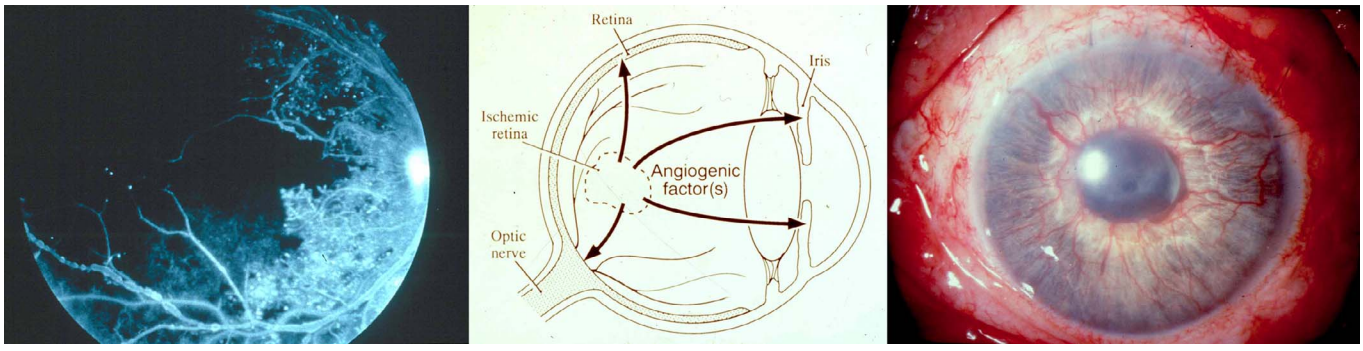

Figure 2. The search for Factor X. *Left*: Fluorescein angiogram of the retina in a patient with diabetic retinopathy (JWM patient seen at Massachusetts Eye and Ear). *Black area* represents nonperfused, ischemic retina. *Center*: Proposed action of unidentified angiogenic factor(s) in ocular neovascularization. Image courtesy of Anthony Adamis, MD; reproduced with permission. *Right*: Color photo of iris neovascularization in a patient with neovascular glaucoma (JWM patient seen at Massachusetts Eye and Ear).

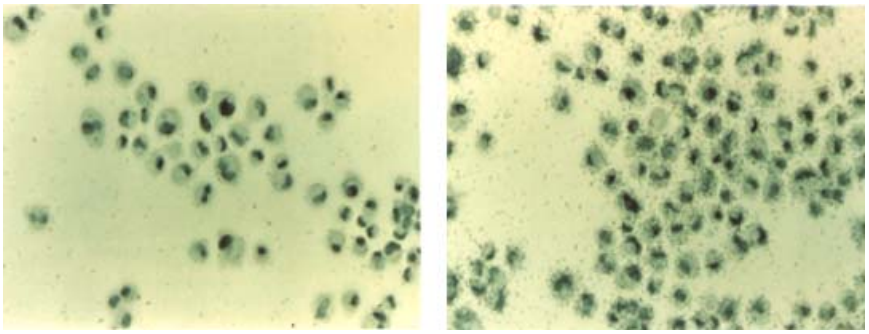

Figure 3. *Left*: Human retinal pigmented epithelial (hRPE) cells hybridized in situ with a VEGF sense riboprobe (control), showing nonspecific cellular labeling and low background levels. *Right*: hRPE hybridized in situ with a VEGF antisense riboprobe, showing strong labeling of all hRPE cells and indicating VEGF expression. Reprinted with permission from Adamis AP, Shima DT, Yeo KT, et al. Synthesis and secretion of vascular permeability factor/vascular endothelial growth factor by human retinal pigment epithelial cells. *Biochem Biophys Res Commun.* 1993;193:631–638. Copyright 1993 Elsevier.[14]

growth, either in the retina, optic nerve, or on the iris (Fig. 2). However, the exact identity of Factor X would remain elusive for several decades.

In the 1970s, Folkman's laboratory identified tumor angiogenesis factor,[4] and Folkman published his seminal theory of tumor angiogenesis in the November 1971 issue of *New England Journal of Medicine*: that angiogenesis, or the recruitment and growth of new blood vessels, was required for tumor growth.[5] However, Folkman's theory was met with skepticism.

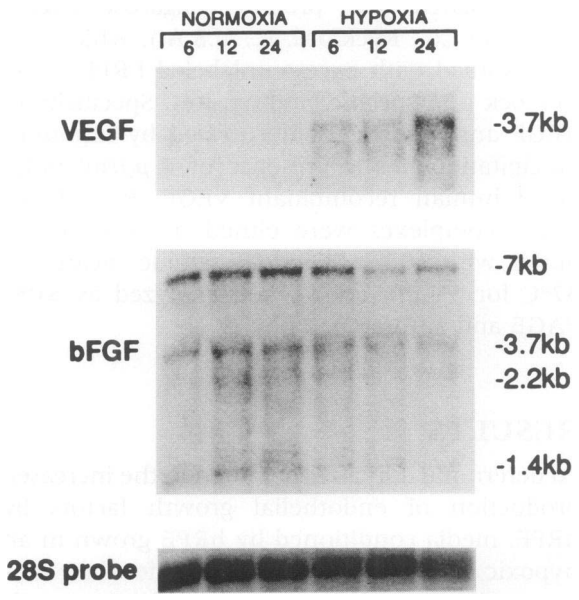

Figure 4. Northern analysis of total RNA extracted from rRPE cells grown under normoxic and hypoxic conditions for 6, 12, and 24 hours, probed for VEGF (*upper*), bFGF (*middle*), and 28S RNA (*lower*). Reproduced with permission from Shima DT, Adamis AP, Ferrara N, et al. Hypoxic induction of endothelial cell growth factors in retinal cells: identification and characterization of vascular endothelial growth factor (VEGF) as the mitogen. *Mol Med.* 1995;1:182–193.[15]

In the 1980s, Pat D'Amore, Bert Glaser, Arnall Patz, and others looked for an angiogenic factor in the retina and vitreous. Fibroblast growth factors 1 and 2 (FGF-1 and FGF-2) were identified as angiogenic factors and potential candidates for Factor X.[6–10] Importantly, however, FGF did not meet all the criteria for Factor X; namely, it is not secreted. The identity of Factor X remained elusive.

In 1983, Harold Dvorak and Don Senger at Harvard Medical School isolated VPF from ascites fluid and tumor cells, and they demonstrated that it was a very potent permeability factor: 50,000 more potent than histamine.[1] In 1989, Napoleone Ferrara and others cloned, sequenced, and characterized VEGF,[2] which turned out to be the same molecule as VPF.[11] VEGF was a secreted endothelial cell mitogen and an angiogenesis factor that was regulated by hypoxia (a condition known to stimulate neovascularization in certain retinopathies). Ferrara identified high-affinity tyrosine kinase receptors for VEGF,[12] and demonstrated that heterozygous *Vegf* knockouts were embryonically lethal.[13]

Intrigued by these findings, our groups set off in two directions to investigate the role of VEGF in ocular disease. First, Adamis et al.[14] grew retinal pigment epithelium (RPE) cells in culture and showed that they indeed produced VEGF (Fig. 3), the first demonstration that ocular cells made this factor. They also demonstrated that this production was regulated by hypoxia (Fig. 4).[15,16]

At the same time, we collected ocular fluid samples from a model of ischemic retinopathy in a nonhuman primate, in which experimental retinal ischemia following laser vein occlusion leads to iris neovascu-

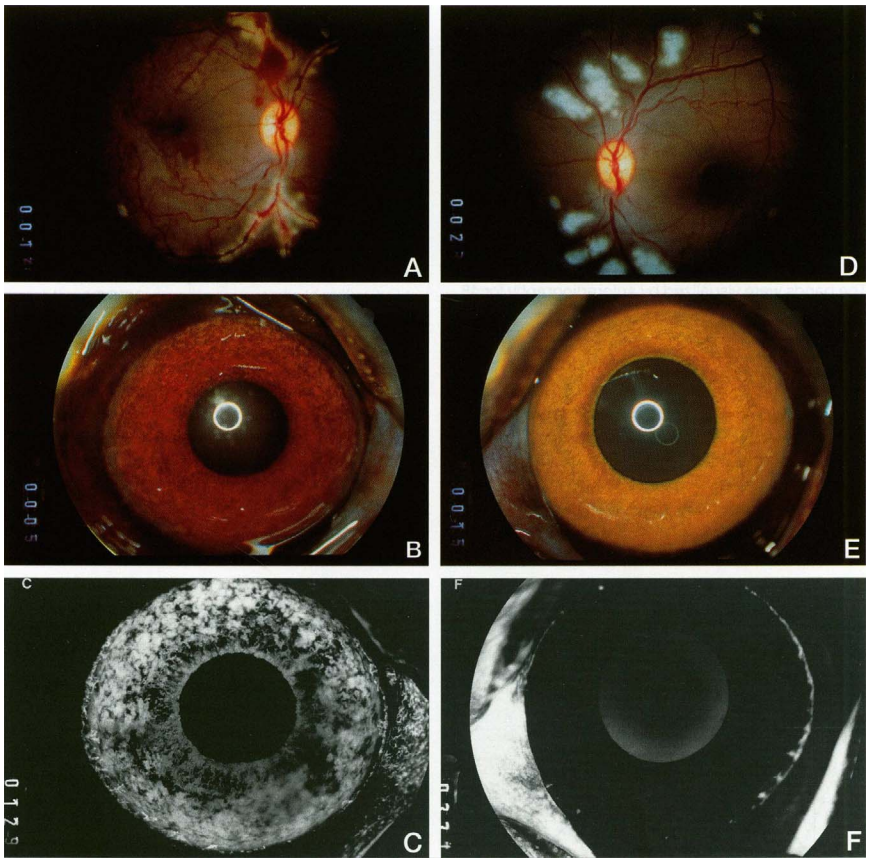

Figure 5. Experimental iris neovascularization. (A) Fundus photograph immediately following laser vein occlusion. (B) Color photograph showing new vessels on the surface of the iris, which appear 4 to 7 days after laser vein occlusion. (C) Fluorescein angiography showing iris neovascularization with abundant leakage of fluorescein (grade 3). (D) Fundus photograph immediately following sham laser, aimed adjacent to the retinal vessels and producing retinal injury but preserving normal vasculature. (E) Color photograph of iris 12 days after sham laser, which appears normal. (F) Fluorescein angiography of the iris in (E), showing normal iris vessels with no fluorescein leakage (grade 0). Reproduced with permission from Miller JW, Adamis AP, Shima DT, et al. Vascular endothelial growth factor/vascular permeability factor is temporally and spatially correlated with ocular angiogensis in a primate model. *Am J Pathol*. 1994;145:574–584. Copyright 1994 Elsevier.[17]

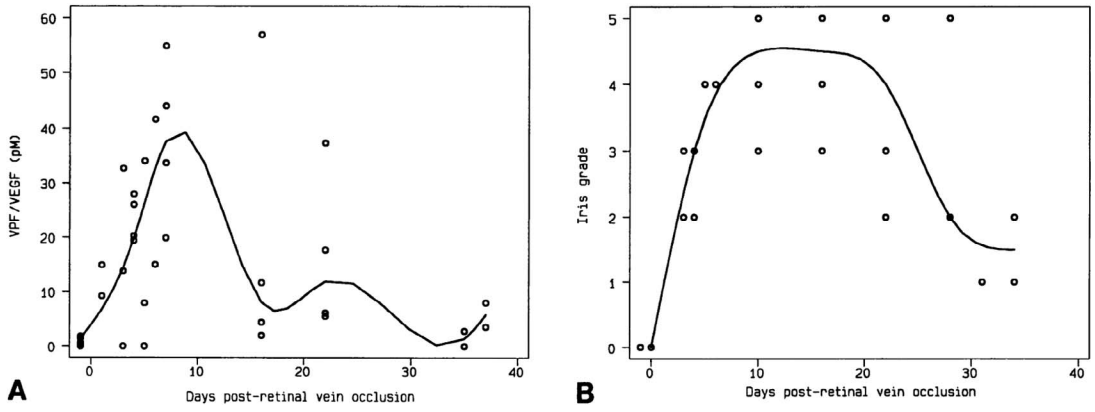

Figure 6. Correlation of VEGF levels in the aqueous (A) and grade of iris neovascularization (B) of four monkey eyes after laser vein occlusion. VEGF levels and neovascularization are represented as a scatterplot with best fit curves. Reproduced with permission from Miller JW, Adamis AP, Shima DT, et al. Vascular endothelial growth factor/vascular permeability factor is temporally and spatially correlated with ocular angiogensis in a primate model. *Am J Pathol.* 1994;145:574–584. Copyright 1994 Elsevier.[17]

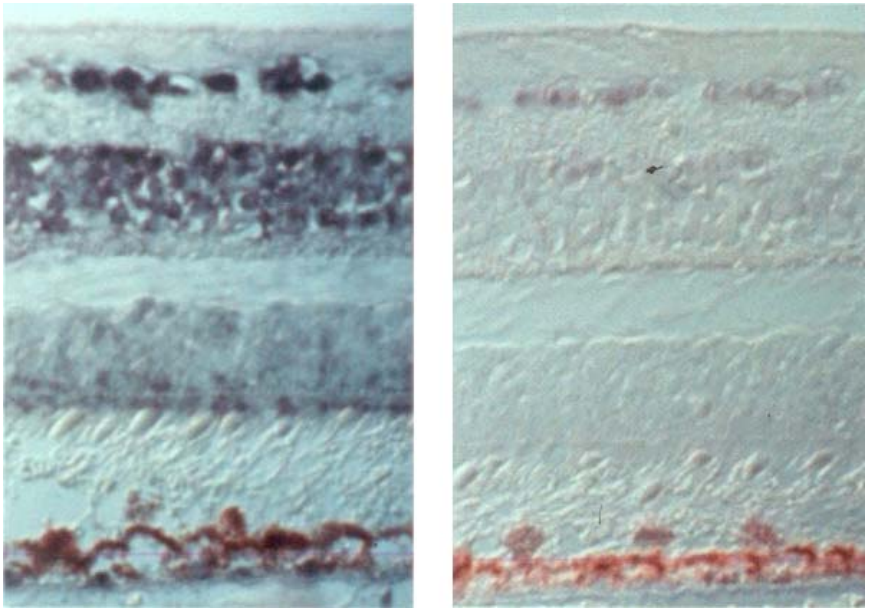

Figure 7. In situ localization of VEGF mRNA in ischemic retinas. *Left*: Cellular localization of VEGF mRNA expression by hybridization with an antisense VEGF riboprobe 13 days after laser vein occlusion. *Right*: Sense (control) riboprobe hybridized in 13-day ischemic retina. Adapted with permission from Shima DT, Gougos A, Miller JW, et al. Cloning and mRNA expression of vascular endothelial growth factor in ischemic retinas of Macaca fascicularis. *Invest Ophthalmol Vis Sci.* 1996;37:1334–1340.[18]

larization (Fig. 5). When we looked at VEGF/VPF levels with Dvorak, we saw that the protein was virtually nonexistent before neovascularization appeared, but rose very quickly to high levels that correlated closely with increasing severity of iris neovascularization, and levels decreased as the iris neovascularization regressed (Fig. 6).[17] This was the first correlation of increased VEGF levels with ocular neovascularization in vivo. We also demonstrated that it was the ischemic retina that produced VEGF,

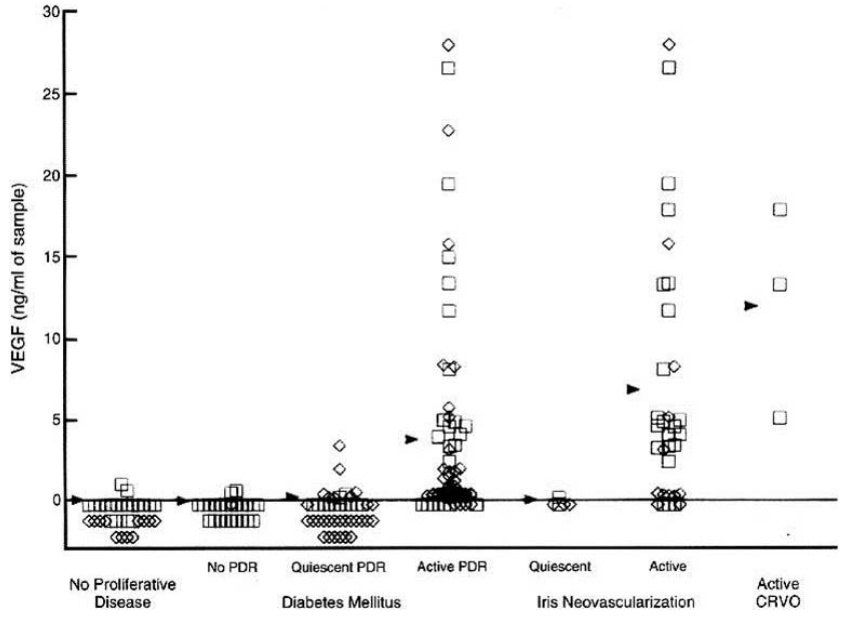

Figure 8. VEGF concentrations in the aqueous (*squares*) and vitreous (*diamonds*) of patients undergoing intraocular procedures. *Arrowheads* indicate mean values. Reproduced with permission from Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med.* 1994;331:1480–1487. Copyright 1994 Massachusetts Medical Society.[20]

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