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Remembrances of things past

The Harvard angiogenesis story☆

*Joan W. Miller, MD**

Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA, USA

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ABSTRACT

I shall discuss the work of researchers at Harvard Medical School who came together in the early 1990s. Scattered across various Harvard-affiliated hospitals and research centers, these individuals were unified by their interest in ocular neovascularization. Together and separately, they investigated models of ocular neovascularization, exploring tumor angiogenesis in eye development and disease.

At Boston Children's Hospital, Judah Folkman instigated the investigation of ocular angiogenesis as an extension of his work in tumor angiogenesis. At Beth Israel Deaconess Medical Center, Harold Dvorak and Don Senger identified a soluble factor known as vascular permeability factor (VPF).[28] Later, this factor was independently identified as vascular endothelial growth factor (VEGF) by Napoleone Ferrara at Genentech.[23] At Massachusetts Eye and Ear Infirmary, Evangelos Gragoudas and Donald D'Amico provided clinical insight and leadership. Across Harvard, there were two primary groups investigating VEGF and ocular angiogenesis. Patricia D'Amore, Anthony Adamis, David Shima, and I formed one at Boston Children's Hospital and the Massachusetts Eye and Ear Infirmary. The other was composed of Lloyd Paul Aiello and George King at Joslin Diabetes Center, later joined by Lois Smith of Boston Children's Hospital. Robert D'Amato led an

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* Corresponding author: Joan W. Miller, MD, Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114, USA.
E-mail address: joan_miller@meei.harvard.edu.

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additional group at Children's, pursuing other aspects of angiogenesis. There were, of course, many collaborators, postdoctoral fellows, and students, without whom the investigations could not have been carried out.

The search for a secreted factor that stimulated neovascularization dates back to 1948, when Michaelson[24] postulated that a soluble and diffusible growth factor (which became known as the mysterious Factor X) was responsible for retinal vascular growth in development and disease. In the 1970s, Folkman postulated that angiogenesis, or the growth of pathologic new vessels, leads to rapid tumor growth.[14] Folkman's theory was met with skepticism. Interestingly, much of his work relied on eye models.

In the 1980s, Pat D'Amore, while working with Arnall Patz and Bert Glaser at Johns Hopkins University, identified the retina and vitreous as potential sources of angiogenic growth factors.[16–18] D'Amore et al later identified acidic fibroblast growth factor (aFGF or FGF-1) as an angiogenic factor and potential candidate for Factor X in ocular neovascularization.[9] Over the next several years, several angiogenic factors were identified.[15] When basic FGF (bFGF) was found to be elevated in vitreous samples of patients with diabetic retinopathy,[32] it became one of the leading candidates for Factor X. Importantly, however, bFGF is not secreted. The identity of Factor X remained unclear.

Meanwhile, Harold Dvorak and Don Senger at Harvard Medical School had isolated VPF from ascites fluid and tumor cell lines and showed that it was 50 times more potent than histamine as a permeability agent.[28] The potent angiogenic effects of VPF, however, were not appreciated until 1989, when Napoleone Ferrara of Genentech identified the molecule that he dubbed VEGF because it led to proliferation of endothelial cells.[23] This proved to be the same molecule as VPF.[21] Several features made VEGF stand out among other candidates for Factor X. In addition to angiogenesis, VEGF also induced vascular leakage, another feature of several neovascular retinal disorders. Importantly, VEGF was secreted in response to and regulated by hypoxia, a condition believed to stimulate neovascularization in many retinopathies.

At Folkman's suggestion, our group initiated some experiments to investigate the role of VEGF in ocular disease, in collaboration with Dvorak's team. Adamis, Shima, and D'Amore first showed that retinal pigment epithelium (RPE) cells produced VEGF in vitro[3] and that this production was regulated by hypoxia.[29,30] At the same time, we began collecting aqueous samples from monkey eyes with experimental retinal ischemia following laser vein occlusion and demonstrated that VEGF levels correlated with the degree of neovascularization—the first correlation of VEGF with ocular angiogenesis in vivo.[25] Using the same model, we were able to demonstrate that VEGF expression occurred in the inner retina, which was producing the secreted VEGF121 and VEGF165 isoforms.[31]

After associating VEGF with neovascularization in monkeys, Adamis and I (in collaboration with D'Amico and Folkman) looked to see whether we could associate VEGF with human ocular disease. We collected vitreous samples from patients and found that VEGF levels were significantly higher in eyes with proliferative diabetic retinopathy than those without.[1] At the same time, Aiello, working with Ferrara and a larger collaborative group, collected aqueous and vitreous specimens from patients with diabetic retinopathy and retinal vein occlusion, including specimens obtained before and after treatment.[4] This study provided the strongest evidence thus far that VEGF protein levels correlated with active proliferative disease in human eyes.

Joined by Smith et al, Aiello then tested soluble VEGF receptors as an inhibitor in an oxygen-induced retinopathy model and showed a significant decrease in retinal neovascularization.[5] Adamis and I tested intravitreal monoclonal antibodies against VEGF (the precursor to Avastin) in the ischemic retinopathy and iris neovascularization model and demonstrated complete suppression of neovascularization.[2] We also showed that injection of VEGF into normal eyes was sufficient to produce iris neovascularization and neovascular glaucoma,[35] as well as retinal ischemia and microangiopathy,[36] in our primate model.

By 1996, we were ready to develop a clinical treatment. We had extensive data that VEGF played a key role in ischemic retinopathy in relevant animal models and clinical disease. A causal role for VEGF in neovascular age-related macular degeneration (AMD) was not proven, but an association was evident. Genentech, which had proven inhibitors of VEGF, had many drugs in their pipeline and were less driven to pursue ophthalmologic indications. Roche was also a large shareholder of Genentech and already been down the AMD path with interferon in the early 1990s.[12,27] Having shown no effect after $18 million was invested in clinical trials, they were not keen to pursue an AMD indication. This combination of factors led to 5 years of inconsistent effort at Genentech, during which they would develop a focused program and then drop it—or consider licensing to another company then choose instead to develop anti-VEGF therapy themselves. Not until several Genentech employees left and licensed an anti-VEGF aptamer and started Eyetech did Genentech really get serious about ophthalmic clinical trials.

Our group continued some work with Genentech, investigating the localization of anti-VEGF antibodies. Concerned that antibodies might not cross the retina from the intravitreal space to affect RPE and choroidal neovascularization (CNV), we tested whether antibodies given systemically would extravasate from experimental CNV. Michael Tolentino, working with Adamis and me, found that intravenous fluoresceinated anti-VEGF antibody (essentially Avastin) reached CNV and persisted in the extravascular space around CNV for up to 5 days.[34]

Genentech proceeded with the development of Avastin and Lucentis, modifying the antibody to increase its affinity for VEGF and its similarity to human antibodies. Magdalena Krzystolik, Adamis, and I then showed the efficacy of intravitreal anti-VEGF antibody fragment (essentially Lucentis) in inhibition of experimental CNV.[22] The experiments using 500 μg were published, but the 2 mg dose was also safe and effective.

Anti-VEGF therapy proceeded into clinical trials. Phase 3 studies were published—first with Macugen showing efficacy, but limited visual results.[19] The phase 3 studies using Lucentis showed truly remarkable results, with more than 90% of

patients preserving vision, one-third improving by three lines or more, and more than one-third retaining vision of 20/40 or better. More recently, the CATT trials showed comparable vision results between Lucentis and Avastin,[8] and VEGF-TRAP (aflibercept, or Eylea) is an emerging anti-VEGF therapy that was approved for use in wet AMD in November 2011.[20]

What about retinal ischemia and macular edema? After all, the early studies showed the strongest correlation of VEGF with retinal ischemia and neovascularization. VEGF does indeed induce retinal vessel changes and causes breakdown of the blood retinal barrier.[37] With the publications of multiple studies in recent years, including the DRCRnet,[10,11] the RISE/RIDE trials,[26] and the phase 3 Lucentis trials,[6,7] we can conclude that anti-VEGF therapy has a clear role in diabetic retinopathy and retinal vein occlusion (RVO). Indeed, Lucentis was approved by the FDA for macular edema following RVO in June 2010 and diabetic macular edema in July 2012; Eylea was approved for macular edema secondary to RVO in September 2012. In addition, both are being investigated for a growing list of ocular conditions, including retinopathy of prematurity, juxtafoveal telangiectasia, radiation retinopathy, and glaucoma.[33] Clearly, VEGF and anti-VEGF approaches are firmly established in ophthalmology. As Napoleone Ferrara has stated, although the majority of angiogenesis research focuses on cancer, the results obtained in noncancerous disorders (such as AMD, macular edema from RVO, and diabetic retinopathy) represent "the most compelling benefits of anti-angiogenic therapy."[13]

It is fun to review the achievements of these researchers and their many collaborators, but what is next? To be successful, one has to mentor successfully, and of course this applies more broadly than any single ophthalmology department. Within our department, we strive to continually grow and guide the next generation of clinician scientists, educators, and researchers. With our colleagues around the globe, we endeavor to find new cures for patients with blinding eye disease so that children born today may see throughout their lives.

We are all truly privileged to work in the field that we do. As Henkind et al wrote in their preface to *The Retinal Circulation*:[38]

"What glories lie within the eye."

Disclosures

Dr. Miller is a co-inventor of U.S. patents and patent applications directed to the use of verteporfin, particularly for the selective destruction of subretinal choroidal neovasculature for the treatment of macular degeneration and other disorders. Massachusetts Eye and Ear Infirmary receives royalties as a result of these patents and patent applications, and Dr. Miller receives a share of the same in accordance with institutional royalty-sharing provisions. Dr. Miller previously held board membership and equity in Alcon, Inc., and continues in a consulting capacity. Dr. Miller also serves as a consultant for Imagen Biotech, Inc.; ISIS Pharmaceuticals, Inc; Kalvista Pharmaceuticals, Ltd; Regeneron Pharmaceuticals, Inc.; and ONL Therapeutics, LLC.

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