

Ocular Angiogenesis as an Organizing Principle in Biomedicine

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BY JUDAH FOLKMAN, MD

STATEMENT OF NEED

Experimental ocular angiogenesis was important in initiating the field of angiogenesis research, and it still contributes to progress in the field today. Models of ocular angiogenesis continue to be fundamental to the growing understanding of a variety of angiogenesis-dependent diseases.

As a result, what ophthalmologists are currently learning about ocular neovascularization, especially in the use of drugs that inhibit certain growth factors in the eye, informs scientists and clinicians across a broad landscape of medicine.

This article reviews some of what we have learned to date about angiogenesis and ends with a look at possible future applications of angiogenesis research – including the possibility of a blood test to predict the recurrence of ocular angiogenesis, which might assist clinicians in the management of age-related macular degeneration (AMD) and other ocular neovascular diseases.

TARGET AUDIENCE

This activity is designed for retinal specialists and other ophthalmologists.

LEARNING OBJECTIVES

Upon successful completion of this learning program, the participant should be able to:

- Review what has been learned to date about angiogenesis.
- Discuss possible future applications of angiogenesis research.
- Cite Judah Folkman's early contributions to the field of angiogenesis investigation.
- Discuss the importance of the corneal neovascularization model.
- Cite the major advances in the development of angiogenesis inhibitors.

METHOD OF INSTRUCTION

Participants should read the learning objectives and continuing medical education (CME) program in their entirety. After reviewing the material, they must complete the self-assessment test, which consists of a series of multiple-choice questions. This test is available exclusively online, at www.CMEToday.net. Once you register and log in, you can take the test, get real-time results, and print out your certificate. Please e-

mail ckoury@bmcctoday.com or call 484-581-1821 if you have any questions or technical problems with the Web site.

Upon completing the activity and achieving a passing score of $\geq 70\%$ on the self-assessment test, participants can print out a CME credit letter awarding *AMA/PRA Category 1 Credit*.™ The estimated time to complete this activity is 1 hour.

ACCREDITATION

This activity has been planned and implemented in accordance with the Essentials and Standards of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of The Dulaney Foundation and RETINA TODAY.

The Dulaney Foundation designates this educational activity for a maximum of 1 *AMA/PRA Category 1 Credit*.™ Physicians should only claim credit commensurate with the extent of their participation in the activity.

DISCLOSURE

In accordance with the disclosure policies of The Dulaney Foundation and to conform with ACCME and FDA guidelines, all program faculty are required to disclose to the activity participants: (1) the existence of any financial interest or other relationships with the manufacturers of any commercial products/devices, or providers of commercial services that relate to the content of their presentation/material or the commercial contributors of this activity; and (2) identification of a commercial product/device that is unlabeled for use or an investigational use of a product/device not yet approved.

FACULTY CREDENTIALS

Judah Folkman, MD, is Professor of Cell Biology at Harvard Medical School and is Director of the Vascular Biology Program at Children's Hospital Boston.

Dr. Folkman graduated cum laude from The Ohio State University in 1953. He continued his education at Harvard Medical School, where he graduated magna cum laude in 1957. Folkman began his surgical residency at Massachusetts General Hospital and served as chief resident in surgery from 1964-1965.

As a student, Folkman co-authored papers describing a new method of hepatectomy for liver cancer and developed the first atrio-ventricular implantable pacemaker for which he received the Boylston Medical Prize, Soma Weiss Award and Borden

Undergraduate Award in Medicine.

While serving as a lieutenant in the U.S. Navy from 1960-1962, Folkman and a colleague at the National Naval Medical Center, Bethesda, MD, first reported the use of silicone rubber implantable polymers for the sustained release of drugs. Their findings became the basis for development of Norplant, the contraceptive used internationally, and initiated the field of controlled release technology. At this time, Folkman also began growing tumors in isolated perfused organs, which led to the idea that tumors are angiogenesis-dependent.

In 1971 Folkman published a seminal paper in the *New England Journal of Medicine*, proposing the hypothesis that all tumor growth is angiogenesis-dependent. This founded the field of angiogenesis research and opened a field of investigation now pursued by scientists in many fields worldwide. Dr. Folkman's laboratory purified the first angiogenic protein from a tumor, discovered the first angiogenesis inhibitors and initiated clinical trials of antiangiogenic therapy. Today, angiogenesis inhibitors have received US Food and Drug Administration (FDA) approval for cancer and for the treatment of AMD and are also approved in 27 other countries. Largely because of Dr. Folkman's research, the possibility of antiangiogenic therapy is now on a firm scientific foundation, not only in the treatment of cancer, but of many nonneoplastic diseases as well.

Dr. Folkman is the author of 389 original peer-reviewed papers and 106 book chapters and monographs. He also holds honorary degrees from fifteen universities and is the recipient of numerous national and international awards. He has been elected to the National Academy of Sciences, the American Academy of Arts and Sciences, the American Philosophical Society and the Institute of Medicine of the National Academy of Sciences.

In addition to his distinguished accomplishments in research, Dr. Folkman has served as a surgeon and teacher. He began his career as an instructor in surgery for Harvard's Surgical Service at Boston City Hospital Boston, was promoted to Professor of Surgery at Harvard Medical School, and became the Julia Dyckman Andrus Professor of Pediatric Surgery in 1968. From 1967 he served as Surgeon-in-Chief at the Children's Hospital Boston for 14 years.

FACULTY DISCLOSURE DECLARATIONS

None.



Figures 1 and 2. In 2007, four reports show that one ligand for the Notch receptors, delta-like ligand 4 (DLL4), is normally induced by VEGF and is a negative-feedback regulator that restrains vascular sprouting and branching. Photos from *Nature*, 1976.

INTRODUCTION

Adapted from an invited lecture at the annual meeting of the Association for Research in Vision and Ophthalmology, May 6, 2007, Fort Lauderdale, Fla.

Experimental ocular angiogenesis was important in initiating the field of angiogenesis research, and it still contributes to progress in the field today. Models of ocular angiogenesis continue to be fundamental to our growing understanding of a variety of angiogenesis-dependent diseases.

As a result, what ophthalmologists are currently learning about ocular neovascularization, especially in the use of drugs that inhibit certain growth factors in the eye, informs scientists and clinicians across a broad landscape of medicine.

This article reviews some of what we have learned to date about angiogenesis and ends with a look at possible future applications of angiogenesis research—including the possibility of a blood test to predict the recurrence of ocular angiogenesis, which might assist clinicians in the management of AMD and other ocular neovascular diseases.

Due to space constraints, this article reviews only selected findings from the more than 30,000 papers that have been published to date on angiogenesis. A recently published review of the subject¹ provides a more complete examination of the literature.

THE EARLY YEARS

Based on experiments in the early 1960s, I reached the conclusion by about 1962 that solid tumors are angiogenesis-dependent. My research suggested that,

to grow beyond microscopic size, tumors needed to recruit new blood vessels.

My papers proposing this hypothesis, however, were rejected many times during the following decade, until one was accepted in 1971.² That paper proposed that solid tumors are dependent on new capillary sprouts, and that without neovascularization these tumors might become dormant, and it predicted the possibility of the future development of angiogenesis inhibitors.

Still, little happened in the field for another 10 years. In 1971, approximately 4,200 papers per week were published in the field of biomedicine, there were three papers on angiogenesis: two from our laboratory and a third criticizing our original paper. That ratio continued for the next 10 years. But starting in 1983, acceptance of the concept of angiogenesis began to grow, and the volume of papers increased. As of April 2007, 30,663 papers have been published in this field of study, with 50 or more new publications every week.

During the 1970s our laboratory developed a series of bioassays for angiogenesis. The most important was the corneal neovascularization model,³ which has since been used in the development of every antiangiogenic drug that has been approved by the FDA.

With the rabbit cornea model of neovascularization we showed that, when tumor cells were implanted in a corneal pocket, new microvessels grew toward the tumor. The tumor then became vascularized and expanded exponentially. This work demonstrated that a factor from or induced by the tumor could diffuse through the cornea and stimulate blood vessel growth.



Figures 3 and 4. As each capillary sprout arises from the same venule, . . . flanking endothelial cells become refractory to new sprout formation. This results in spacing between sprouts.

Robert Langer in our laboratory developed implantable pellets of poly-hydroxyethyl methacrylate that could provide sustained release of a proangiogenic factor in the cornea.⁴ These pellets caused new vessels to sprout from the corneal limbus (Figures 1-4). At that time, it was noted that the new sprouts were evenly spaced, with nonvascularized gaps between them. This spacing phenomenon has never been explained: If the whole length of the vessel is stimulated by an angiogenic factor, why are there gaps? But four papers published in recent months may help to explain the mechanism.⁵⁻⁸

A newly identified molecule, delta-like ligand 4, which is normally induced by vascular endothelial growth factor (VEGF), is a negative feedback regulator that suppresses the growth of vessels. In a mouse model, when the gene for delta-like ligand 4 was knocked out, vessels sprouted in a bush-like form without spacing. This recent work by multiple centers illustrates how ocular angiogenesis models continue to contribute to our understanding of the field.

Early work by our laboratory with the rabbit model also showed that, when the angiogenic stimulus was removed by explanting the implantable pellet, neovascularization regressed over time.⁹ This gave us confidence that, if an angiogenesis inhibitor could be developed, vessel growth in tumors could possibly regress and disappear.

Using the bioassays developed in our lab, especially the corneal neovascularization model, we identified 12 angiogenesis inhibitors over a period of 25 years, beginning with low-dose interferon alpha in 1980.¹⁰ Eight of these inhibitors were found in the body; these endogenous angiogenesis inhibitors are nontoxic.

There are now 29 known angiogenesis inhibitors, some of them very potent.

PHARMACEUTICAL DEVELOPMENT

Eventually the efforts of our lab and others in the identification of angiogenesis inhibitors began to catch the attention of the pharmaceutical industry. If we fast-forward to June 2007, 10 angiogenesis inhibitors are now approved by US regulators, and antiangiogenic drugs have received approvals in more than 30 other countries.

Bortezomib (Velcade; Millennium, Cambridge, MA) was approved for treatment of multiple myeloma in 2003. It was not known to be antiangiogenic until after it was approved. Bevacizumab (Avastin; Genentech, San Francisco) was approved for the treatment of colorectal cancer in 2004. Erlotinib (Tarceva; OSI Pharmaceuticals, New York, NY), also approved in 2004, is now known to block three angiogenic factors.

Pegaptanib sodium (Macugen, OSI/Eyetech, New York, NY) was the first angiogenesis inhibitor to be FDA-approved for treatment of age-related macular degeneration, in December 2004. Ranibizumab (Lucentis, Genentech) followed in June 2006.

The rate of US and international approvals and labeling extensions continues to increase. (As an example of labeling extensions, Avastin is now approved for the treatment of lung cancer in the United States and for metastatic breast cancer in the countries of the European Union.)

The pipeline of drugs in development also continues to grow. As of February, there were more than 20 angiogenesis inhibitors, mostly targeting VEGF or VEGF receptors, in phase 3 clinical trials. More than 50

compounds are in phase 2 trials.

The drugs further back in the pipeline target a broad range of molecules, not only VEGF and pigment-derived growth factor (PDGF) and their receptors, but a number of compounds that most ophthalmologists are not yet familiar with. Some of these drugs block more than one proangiogenic protein. This robust pipeline indicates that, in the very near future, a wide variety of antiangiogenic drugs may be introduced to ophthalmology, not all of them targeting only VEGF.

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Oncologists are currently using many of these drugs in combinations. If current clinical trials for diabetic retinopathy reveal that retinal angiogenesis in diabetes is more exuberant than choroidal angiogenesis in AMD, it may be necessary to use combinations of angiogenesis inhibitors for that indication.

Most of the existing drugs are indirect angiogenesis inhibitors; that is, they block the expression of a proangiogenic protein, they block the protein on the way to an endothelial cell receptor, or they block the receptor for that protein. They are called indirect because they block the products of tumor cells or the receptors for those products. The next generation of drugs may include direct angiogenesis inhibitors—agents that directly turn off pathologically activated endothelial cells and prevent them from migrating toward a tumor. These direct inhibitors tend to have a broad spectrum of antiangiogenic activity and low toxicity.

NEOVASCULAR AMD

In ophthalmology, it was long suspected that some sort of diffusible factor coming from the back of the eye was mediating neovascular AMD, but the molecular nature of this “X factor” was unclear until the 1990s. Retina subspecialists who now use antiangiogenesis drugs on a routine basis may be interested in how VEGF came to be identified.

In 1983, Senger and colleagues described a tumor-

derived vascular permeability factor (VPF) that caused ascites.¹¹ It was not known at that time to be an endothelial growth factor. Our laboratory then reported the purification of basic fibroblast growth factor (bFGF) from a tumor, the first use of heparin affinity chromatography to purify an angiogenic factor.¹²

In the late 1980s, Ferrara and Henzel at Genentech used the heparin binding affinity technique to isolate a different growth factor from a pituitary cell. At the same time, our laboratory had also purified a second endothelial growth factor from sarcoma 180 cells. I received a telephone call from Napoleone Ferrara. He had heard that we had purified another endothelial growth factor, and he told me he also had purified one. He suggested that we exchange factors, which we did. Ferrara compared the amino acid sequence of the two factors and determined that they were identical. This was fortunate for us because at the time we were more than a year away from accumulating sufficient purified protein to determine the amino acid sequence of our factor.

Ferrara and Henzel’s paper on this second endothelial growth factor appeared in 1989.¹³ Our paper appeared in 1990, with Ferrara and Henzel listed as coauthors because they had done the sequencing for us.¹⁴ VPF also turned out to be the same as VEGF.

In the early 1990s, our laboratory began to look at the role of this *vascular permeability factor*—now better known as VEGF—in ocular neovascularization. Anthony Adamis (at the time an assistant professor in my laboratory; he is a cofounder of Eyetech Pharmaceuticals) and coworkers reported that VEGF was secreted by retinal pigment epithelial cells.¹⁵ Then, in collaboration with Joan Miller lab at the Massachusetts Eye and Ear Infirmary, we reported that VEGF levels were significantly increased in the vitreous of monkey eyes with laser-induced retinal neovascularization.¹⁶

We also reported that high levels of VEGF were found in samples of human vitreous from diabetic eyes.¹⁷ Next, we determined that VEGF expression was increased in retinal cells subjected to hypoxia.¹⁸ Then Ferrara sent us a VEGF antibody—the precursor of bevacizumab and ranibizumab. We showed that this antibody developed by Genentech could inhibit hypoxia-induced iris neovascularization in monkeys.¹⁹

This was a seminal finding—that an antibody to VEGF could be used therapeutically to treat ocular neovascularization in a nonhuman primate. This became the basis for the antiangiogenic therapies used

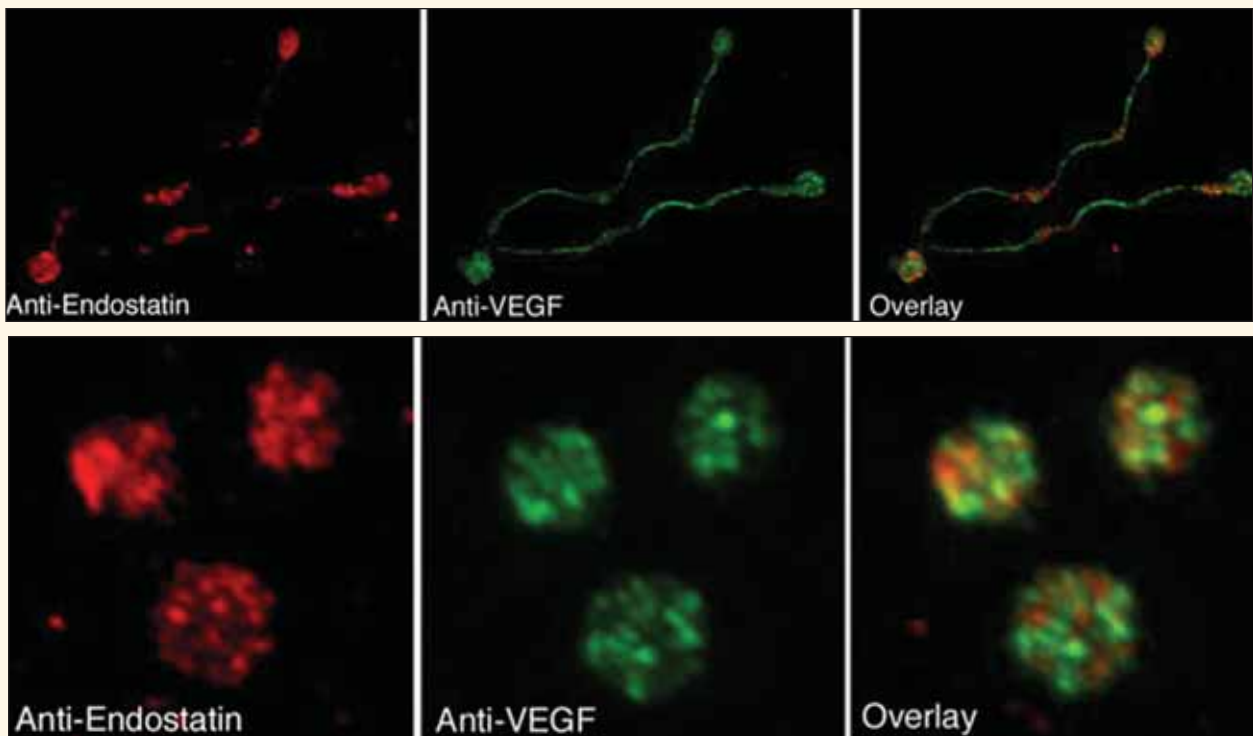


Figure 4. VEGF and endostatin expressed by megakaryocytes, are sequestered in separate compartments in platelets. Two types of alpha granules are now identified: proangiogenic and antiangiogenic. Images from J. Italiano, et al. *Blood*. 2006;108:120a.

worldwide today to treat neovascular AMD and now in clinical trials for the treatment of diabetic retinopathy. It was during this period in the early 1990s when Adamis discovered pegaptanib. And it was from a fragment of the bevacizumab molecule that Ferrara and colleagues developed ranibizumab. These are the two antiangiogenic drugs that are now FDA approved for use in treating neovascular AMD in the United States.

FUTURE DIRECTIONS

What is the next step in angiogenesis research? Our laboratory is currently investigating the possibility of developing a blood test for the recurrence of ocular neovascular diseases such as AMD or diabetic retinopathy. The same type of test is also in development for early detection of cancer recurrence.

This investigation is based on the concept that it may be possible to identify a biomarker in the blood that will predict when choroidal neovascularization or macular edema will recur, after cessation of an antiangiogenic therapy. For instance, in an AMD patient whose vision has been stabilized or improved after a

year's worth of monthly injections of an antiangiogenic agent, such a test could be used to monitor for recurrence and reinstate treatment before vision loss occurs.

Our laboratory is investigating whether the platelet angiogenesis proteome can be used to detect the recurrence of neovascular AMD or diabetic retinopathy.

Our laboratory is investigating whether the platelet angiogenesis proteome can be used to detect the recurrence of neovascular AMD or diabetic retinopathy long before recurrence could be seen on ophthalmoscopy—perhaps years before the recurrence of disease (Figures 4-6). A blood test like this might allow patients to be monitored and treated for recurrent pathology.

While no such test exists yet, research in our laboratory suggests that analysis of platelets can document

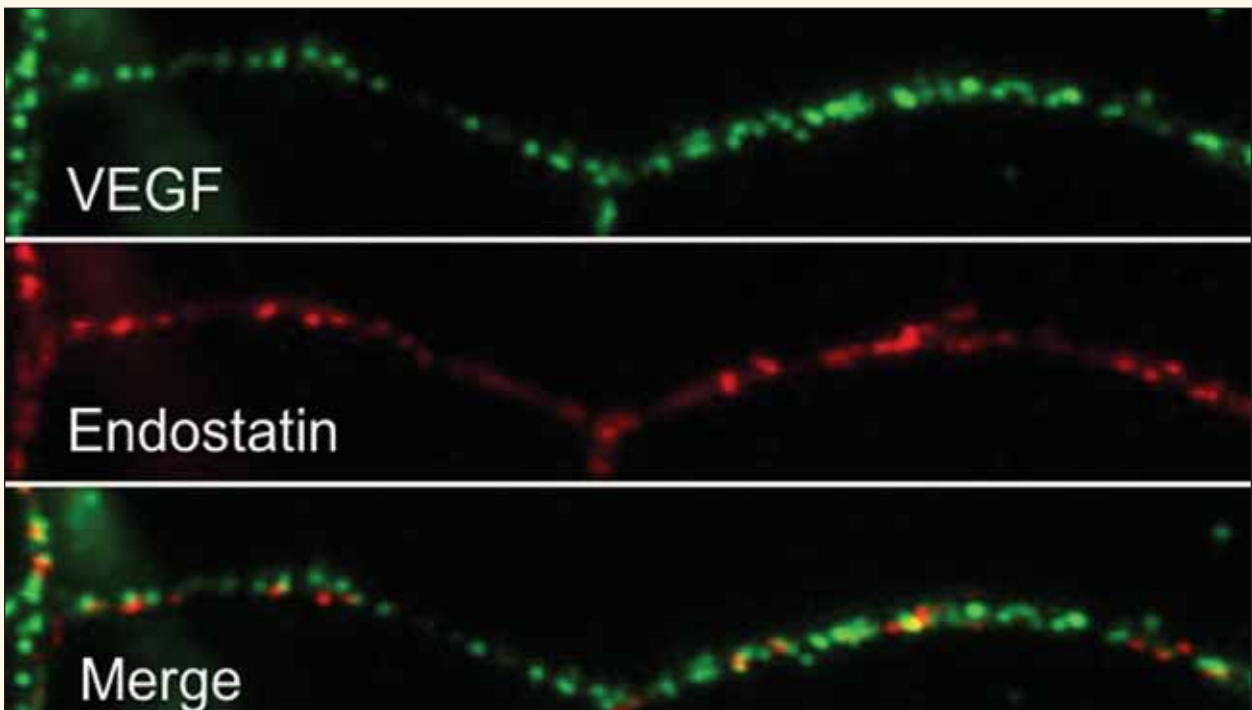


Figure 5. Proplatelets: VEGF and endostatin are stored in separate platelet compartments (mouse platelets). Images from J. Italiano, et al. *Blood*. 2006;108:120a.

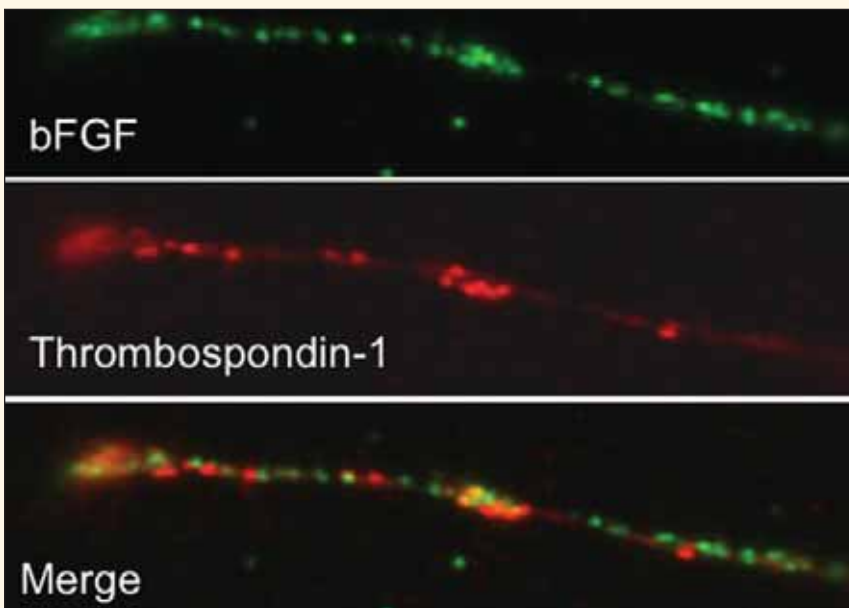


Figure 6. Proplatelets: bFGF and thrombospondin-1 are stored in separate platelet compartments. Images from J. Italiano, et al. *Blood*. 2006;108:120a.

the levels of endogenous proangiogenic and antiangiogenic proteins in circulation. We have found that levels of these angiogenesis proteins inside platelets are sig-

nificantly higher than their levels in plasma or serum, and measurement of them in the platelets more accurately reflects their total concentrations.

Work by Joseph Italiano, PhD, of Brigham and Woman's Hospital, in collaboration with our laboratory, has shown in mice that proangiogenic and antiangiogenic proteins are sequestered in separate alpha-granules within the platelets.²⁰ When the megakaryocytes produce platelets, the proangiogenic proteins are assembled in one alpha-granule and antiangiogenic proteins in a separate alpha-granule. This segregation has implications for how these two types of proteins are released during different conditions, whether for tissue repair or development or in pathologic states such as tumors.

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We have also shown that the platelets take up VEGF. In a mouse implanted with radiologically labeled VEGF, 95% of the radiolabeled VEGF was contained in platelets and none was detected in the plasma.²¹ Verheul and colleagues found that human platelets also take up bevacizumab and sequester it in the alpha granules, where it binds with VEGF and decreases the proangiogenic activity of the platelets.²² Ophthalmologists have observed that some AMD patients, when injected with bevacizumab in one eye, may experience improvement in the contralateral eye. Could the uptake of bevacizumab by platelets be an explanation for this phenomenon? Are platelets carrying the drug systemically to the patient's other eye? Our laboratory is investigating this possibility.

Platelets scavenge VEGF from plasma and platelets maintain concentrations of VEGF many times higher than plasma.

The work just described suggests that platelets may scavenge VEGF from plasma and that platelets maintain concentrations of VEGF many times higher than plasma. Could it be, then, that platelets can be used to detect the presence of a tumor? Do they scavenge from the plasma the angiogenesis regulatory molecules secreted by tumors? Current work in our laboratory suggests that this is the case, and that the platelet angiogenesis proteome may be capable of detecting not only tumor recurrence, but also the recurrence of ocular neovascularization in AMD or diabetic retinopathy.

SUMMARY

The lessons learned from experimental ocular neovascularization provided the basis for the development of antiangiogenic drugs, and they continue to provide new insights for the treatment of cancer and other angiogenesis-dependent diseases. Angiogenesis inhibitors developed to treat cancer have led to the development of similar inhibitors to treat ocular neovascularization, including AMD and diabetic retinopathy.

The platelet angiogenesis proteome is currently in clinical trials for the detection of recurrent microscopic cancers years before symptoms appear and before anatomical location is possible. This methodol-

ogy may also have applications in monitoring patients with AMD. ■

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CME QUESTIONS

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1. The theory that solid tumors are dependent on neovascularization, and that without new capillary sprouts these tumors might become dormant, was first published in
 - a. 1962
 - b. 1971
 - c. 1989
 - d. 1996
2. The cornea neovascularization model that has been used in the development of all antiangiogenic drugs to date used what animal?
 - a. mouse
 - b. rat
 - c. rabbit
 - d. monkey
3. The antiangiogenic drugs currently approved for treatment of AMD target which growth factor(s)?
 - a. PDGF
 - b. VEGF
 - c. bFGF
 - d. all of the above
4. The work that identified VEGF as important in ocular neovascularization took place in what period of time?
 - a. the late 1970s
 - b. the late 1980s
 - c. the early 1990s
 - d. the early 2000s
5. Most existing antiangiogenic drugs are indirect angiogenesis inhibitors. This means
 - a. they block the expression of a proangiogenic protein
 - b. they block the protein on the way to an endothelial cell receptor
 - c. they block the receptor for that the protein
 - d. any one of the above
6. Proangiogenic and antiangiogenic proteins are found in higher concentrations in
 - a. platelets
 - b. serum
 - c. plasma
 - d. whole blood
7. Platelets can sequester
 - a. proangiogenic proteins
 - b. antiangiogenic proteins
 - c. VEGF
 - d. bevacizumab
 - e. all of the above
8. The platelet angiogenesis proteome may be capable of detecting
 - a. the recurrence of tumors
 - b. the recurrence of ocular neovascularization in AMD
 - c. the recurrence of ocular neovascularization in diabetic retinopathy
 - d. the recurrence of elevated systemic hypertension
 - e. a, b, and c