TAMING VESSELS TO TREAT CANCER.

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**Abstract:**

This article updates readers on promising developments in the treatment of cancer. Abnormal and malfunctioning blood vessels are a standard of solid tumors and they contribute to the malignancy of a cancer and prevent treatments from attacking the rogue tumor cells. Researchers found that if they could normalize the blood vessels, the cancer medications would get into the tumors and work more effectively. INSETS: ABNORMAL VESSELS MAKE TROUBLE;Vessel Repair: Beyond Cancer.

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**Restoring order to the chaotic blood vessels inside a tumor opens a window of opportunity for attacking it. Surprisingly, drugs meant to destroy vasculature can make the repairs and may help reverse conditions that lead to cardiovascular disease and blindness**

While still a graduate student in 1974, I had a chance to see malignant tumors from a most unusual perspective. I was working at the National Cancer Institute in the laboratory of the late Pietro M. Gullino, who had developed an innovative experimental setup for studying cancer biology--a tumor mass that was connected to the circulatory system of a rat by just a single artery and a single vein. As a chemical engineer, I decided to use this opportunity to measure how much of a drug injected into the animal would flow to the tumor and back out again. Amazingly, most of the substance injected into the rat never entered the tumor. To make matters worse, the small amount that did reach the mass was distributed unevenly, with some areas accumulating hardly any drug at all.

My immediate concern was that even if a small fraction of the cancer cells in a human tumor did not receive an adequate dose of whatever anticancer drug was being applied, those cells could survive--causing the tumor to grow back sooner or later. Perhaps the engineer in me was also drawn to trying to understand and solve the apparent infrastructure problem inside tumors that posed a major obstacle to the delivery of cancer therapies.

Over the subsequent decades my colleagues and I have investigated what makes the vasculature within tumors abnormal and how these disordered blood vessels not only stymie traditional cancer treatments but also contribute directly to some of the malignant properties of solid cancers. Building on these insights, we developed approaches to normalizing tumor blood vessels and tested them successfully in mice. In the process, we also discovered a seeming paradox--a class of drugs designed to destroy the blood vessels of tumors actually acts to repair them, creating a window of opportunity to attack the cancer most effectively.

In recent years we have finally been able to start testing this idea in cancer patients, and the excitement in our lab was overwhelming when we saw the first clinical evidence of tumors shrinking in response to vascular normalization, just as we had anticipated. Much more work remains before we can perfect this therapeutic approach and gauge its usefulness in patients with different types of malignancy. But what we have already learned about restoring blood vessels is also opening doors to treating other vascular disorders, such as macular degeneration, the leading cause of blindness in the U.S.

**Tortuous Road**

The journey that led to our recent successes began in earnest a few years after I completed my doctoral studies. Determined to find out why drugs do not penetrate tumors uniformly, my colleagues and I started by monitoring every step of the process in rodents. Using a variety of techniques, we observed the progress of drugs as they entered the tiny blood vessels of a tumor, crossed the vessel walls into the surrounding tissue, entered into cancer cells and eventually exited the mass. Together with my students and collaborators, we developed methods for tracking molecules, such as oxygen, within blood vessels and tissues. Eventually we could even watch as genes turned on and off inside cells.

Early on it was apparent that the vessels within tumors bear little resemblance to normal ones. Healthy tissues are fed by straight vessels that branch predictably into successively smaller capillaries and microvessels, creating a pervasive network for delivering oxygen and nutrients to cells. Tumors, which stimulate the growth of new vasculature of their own, tend to generate a tangle of vessels. These connect to one another randomly, with some oversize branches, many extraneous immature microvessels and areas of a tumor that will lack vessels altogether.

Over the course of many years we managed to delineate the processes that govern the movement of fluids, drugs and cells within this tortuous vasculature and gained insight into the consequences of the abnormalities. The picture that emerged was grim: the very first thing we realized was that tumor blood vessels are not just disorganized in their appearance but highly aberrant in every aspect of their structure and function. We found that blood flows quite briskly in some vessels within a tumor, whereas it is static in others. In a given vessel, blood may travel in one direction for a while and then reverse direction. These flow patterns alone create a major obstacle to uniform drug delivery. Moreover, some parts of the vessel walls are overly leaky and others are unusually tight, which means that drugs and other molecules that managed to penetrate the vasculature would be distributed into the surrounding tumor tissue unevenly.

When we began investigating the causes of this nonuniform porousness, we discovered that in some tumors the pores in blood vessel walls could be as large as one or two microns in diameter, which is more than 100 times the size of pores in healthy vessels. As a result, these vessels are unable to maintain normal pressure gradients across their walls. Fluid pressure inside healthy blood vessels is typically much higher than in the surrounding tissue. Because tumor vessels are so porous, escaping fluid raises the outside--or interstitial--pressure until it nearly equals that inside the blood vessels.

This unnatural pressure gradient is not just an impediment to the ability of drugs to reach tumor cells; the accumulation of interstitial fluid produces swelling in and around tumor tissues. In patients with brain cancers, where tissue expansion is limited by the skull, that swelling becomes a severe, often life-threatening problem in itself. In those with other types of cancer, the exuded fluid can also accumulate in body cavities. Wherever it goes, the fluid oozing from a tumor carries with it tumor cells as well as various tumor-generated proteins that promote the growth of new blood and lymphatic vessels in the surrounding normal tissue and lymph nodes--which can then serve as conduits for the metastatic spread of the cancer cells to other parts of the body.

Beyond the difficulty of delivering drugs through chaotic tumor vasculature and the dangerous fluid buildup caused by leaky vascular walls, the abnormalities of tumor vessels create a highly unnatural microenvironment inside a tumor as well. Because many areas of a tumor lack vasculature and existing vessels are unable to deliver sufficient oxygen to surrounding tissues, a general state of hypoxia (low oxygen) and high acidity prevails in the tumor. Hypoxia in turn makes tumor cells more aggressive and prone to metastasis. In addition, the body's immune cells, which might help fight a tumor, are hampered by acidity and cannot function in low oxygen. Nor can radiation treatments and a subset of chemotherapy drugs that depend on chemical processes that require oxygen to kill cancer cells.

Thus, what began as an inquiry into seemingly simple aberrations in the flow of drugs inside tumors revealed that the abnormalities in tumor blood vessels are obstacles to treatment in even more ways than I had initially imagined. In 1994 I described our findings up to that point in-depth in this magazine [see "Barriers to Drug Delivery in Solid Tumors," by Rakesh K.Jain; SCIENTIFIC AMERICAN, July 1994]. By that time, these observations were also beginning to suggest to my research collaborators and me that if we knew how to repair the structure and function of tumor-associated blood vessels, we would have a chance to normalize the tumor microenvironment and ultimately improve cancer treatment. To accomplish such a reversal, we first had to gain a better understanding of what makes tumor vessels abnormal and keeps them that way.

**Restoring Balance**

We began to look at the molecular factors involved in normal blood vessel formation, known as angiogenesis, including the single most potent one, vascular endothelial growth factor (VEGF). First discovered and named vascular permeability factor by my Harvard University colleague Harold Dvorak, VEGF promotes the survival and proliferation of endothelial cells, which form the inner lining of blood vessels. In excess, it also makes vessels leaky-- hence its original name. In normal tissues, however, the collective action of VEGF and other growth-stimulating molecules like it is counterbalanced by the actions of natural antiangiogenesis molecules, such as thrombospondin, that inhibit blood vessel growth.

Whether healthy or diseased, tissues that need new blood vessels increase their production of angiogenesis stimulators or reduce their production of inhibitors, or do both, tipping the balance in favor of angiogenesis. In healthy processes such as wound healing, a balance between growth and inhibitory factors is eventually reinstated once the new vessels are established. But in tumors and a number of other chronic diseases, an imbalance persists--and blood vessels grow increasingly abnormal.

Because VEGF is abundant in most solid tumors, I suspected that finding a way to mop up the excess VEGF or interfere with the growth signals it generates could restore balance and cause tumor vasculature to revert to a more normal state. Alternatively, increasing the concentration of angiogenesis-inhibiting factors could have the same normalizing effect on the blood vessels. I also theorized that vessels treated in either way would not remain normal forever-- they would be destroyed if the inhibitors were potent enough or would become abnormal again if the tumors developed the ability to make different stimulators, such as basic fibroblast growth factor (bFGF), which can mimic many of the effects of VEGF. The only way to find out was to try angiogenesis inhibitors on tumors and see what happened.

In 1995 antibody-based drugs that could neutralize the effects of VEGF were already in development, so we were able to use these to test our approach in mice. Certain of the antibodies attached directly to VEGF, hindering its ability to send a growth signal to endothelial cells by binding to receptors on the cell surface. Other antibodies bound to the VEGF receptors themselves, preventing the growth factor from making contact. Remarkably, both forms of VEGF inhibition caused some of the immature and inefficient blood vessels characteristic of many tumors to be pruned away and induced the remaining vessels to remodel themselves so that they began to resemble normal vasculature. Those normalized blood vessels were less leaky, less dilated and less tortuous. We could also detect functional improvements in the tumors, including lower interstitial fluid pressure, higher oxygenation and improved penetration of drugs.

As excited as we were by these results and by the fact that they were later reproduced in animals by other researchers, we still could not know whether the same responses would occur in cancer patients. And many researchers were understandably skeptical of our approach. By the late 1990s, when I first proposed the idea of tumor vessel normalization publicly, scientists in academia and industry had been working on making drugs to destroy blood vessels. Their pursuit was based on the hypothesis put forward in 1971 by my Harvard colleague Judah Folkman that tumor growth could be halted by starving the mass using antiangiogenic drugs [see "Vessels of Death or Life," by Rakesh K. Jain and Peter Carmeliet; SCIENTIFIC AMERICAN, December 2001]. Indeed, the drug Avastin, approved by the U.S. Food and Drug Administration for use in cancer treatment in 2004, is a VEGF-neutralizing antibody originally developed as such an antiangiogenesis agent.

In laboratory testing and clinical trials, Avastin has been shown to destroy blood vessels in animal and human tumors, although when used alone it does not increase overall survival in cancer patients. In a pivotal clinical trial that led to its approval, however, Avastin did increase the survival of patients with advanced colorectal cancer but only when it was used in conjunction with standard chemotherapy. That positive outcome seemed quite paradoxical at the time because, in principle, a drug that was designed and deployed to destroy blood vessels should reduce the effectiveness of chemotherapy, which requires functioning blood vessels to reach tumor cells. Some published studies have in fact shown that antiangiogenic agents can hinder radiation and chemotherapy. So how could these apparently contradictory findings be reconciled?

Our group had the chance to find out by closely examining the structure and function of blood vessels in the tumors of rectal cancer patients receiving Avastin and combined chemotherapy and radiation in a 2002 clinical trial supported by the National Cancer Institute and led by Christopher Willett, now at Duke University Medical Center. Very quickly, we saw that the changes to tumor vasculature in those patients were not limited to simple vessel destruction.

Two weeks after a single injection of Avastin, blood flow within the tumors did drop by 30 to 50 percent in six consecutive patients. The density of microvessels, the overall number of blood vessels and the interstitial fluid pressure in the tumors were all reduced as well. And a form of programmed cell death known as apoptosis, characteristic of oxygen and nutrient deprivation, increased among tumor cells that no longer had access to the pruned vasculature.

Surprisingly, however, there was no concurrent decrease in a sign of overall energy use by the tumor, its uptake of a glucose analogue, as might be expected if the tumor were only being starved. Instead it seemed that the remaining tumor vessels had become more efficient in supporting the energy needs of the surviving cancer cells. Furthermore, the rate of proliferation of cancer cells increased in some tumors, reflecting their access to better-functioning vessels and a more normal tissue microenvironment. Although increased proliferation is usually not desirable when it comes to cancer cells, that state would make them more sensitive to chemotherapy drugs, which generally target dividing cells.

Together these results provided a first glimpse of how a drug like Avastin works in patients and thereby revealed why it might improve the outcome of radiation or chemotherapy for a time. As the drug blocks the effects of VEGF, some tumor vasculature is pruned away immediately, but the vessels that remain become less abnormal. In addition to improving the overall tumor microenvironment, those normalized vessels also make the surviving cells more vulnerable to the treatments that they can now deliver more efficiently. Restoring normal function to tumor vessels thereby creates a period during which treatment with a variety of cancer therapies should be maximally effective.

**Window of Opportunity**

To truly benefit from this new insight into the way that antiangiogenic therapy can work with radiation or chemotherapies, an oncologist would need to know when a patient's tumor vessels first begin to normalize and how long they remain that way. My research group returned to experimenting with mice to better characterize this period we came to call the "normalization window." We treated brain tumors in the animals with an antibody designed to block the main VEGF receptor used by endothelial cells and saw signs of vessel normalization begin after one day. During the normalization window--which lasted only about five to six days--tumor oxygenation increased and radiation therapy yielded the best therapeutic outcome. Other teams working with laboratory animals have subsequently reported similar observations.

Enough evidence supported this model, in fact, that we were able to test it in another National Cancer Institute clinical trial, completed just over a year ago. Led by my Massachusetts General Hospital colleagues Tracy Batchelor and Gregory Sorensen, the trial included 30 patients whose brain tumors, known as glioblastomas, had re-grown despite aggressive surgery, radiation and chemotherapy. These patients had a life expectancy of less than six months.

They received a daily oral dose of Recentin, an experimental drug that potently inhibits the three primary cellular receptors for VEGF. Using advanced imaging techniques, we were able to look for effects in their tumors and saw them almost immediately [see illustration on opposite page]. The signs of vascular normalization included reduced vessel diameter and leakiness, which continued for at least 28 days, with some normalized characteristics persisting for the entire four-month duration of the study. Moreover, as anticipated by our original model, the normalization was accompanied by a rapid decrease in swelling in and around the tumor, an effect that continued as long as the patients took the Recentin. Because the side effects of VEGF inhibition can be severe, however, some patients asked for a break from the treatment during the trial, which allowed us to observe the tumor vessels becoming abnormal again when Recentin was discontinued, and renormalized when the drug was resumed.

These results were the first to define how long the period of vascular normalization can last in humans and have led to a much larger ongoing clinical trial involving 300 patients to further define the role that Recentin, with and without chemotherapy, might play in the treatment of glioblastoma. We are also studying a number of antiangiogenic drugs in combination with traditional therapies in newly diagnosed and recurrent tumor cases in more types of cancer.

At the same time, we are also investigating ways of expanding the window of normalization so that survival improvements can be extended from months to years. Any potential strategy to repair vessels must recognize that VEGF blockade alone may not always be sufficient to achieve or sustain normalization, because tumors can substitute other growth factors to get around the loss of VEGF signaling. As tumors grow larger, for instance, they tend to make a diverse array of proangiogenic molecules in addition to VEGF, so their vessels may gradually lose responsiveness to a treatment such as Avastin.

In rectal cancer patients, for instance, our group discovered that blood levels of VEGF and P1GF (placental growth factor), a related molecule, actually increased after VEGF was mopped up with Avastin, suggesting that the tumor or other tissues began manufacturing more of those factors in response. And in recurrent glioblastoma patients, blood levels of multiple proangiogenic molecules rose as tumors escaped the Recentin treatment.

This diversification of progrowth signals illustrates that the challenge for the oncologist will be to formulate cocktails of agents specifically tailored to the molecular profile of each patient's primary and metastatic tumors and to changes in those profiles that will likely occur over time. It is worth noting, however, that the available tools for promoting vascular normalization are not limited to drugs targeting VEGF or other growth factors directly. We have shown in mice, for example, that the drug Herceptin-- an antibody that targets a tumor cell-surface protein called HER2 and that is given to about a quarter of women with breast cancer--can mimic the responses produced by an antiangiogenic cocktail and normalize tumor vessels. Herceptin indirectly lowers cellular manufacture of several proangiogenic molecules while increasing the cells' production of the antiangiogenic thrombospondin-1.

In addition to identifying new and existing medications that can foster vascular normalization, it will be important to find minimally invasive and affordable ways for doctors to monitor the normalization process, to best exploit it when delivering treatments. To that end, my colleagues and I have been working to identify so-called biomarkers: readily identifiable signs that reflect what is happening inside the tumor and thereby reveal the onset and duration of the normalization window in individual patients. Such markers might include, for example, proteins in the bloodstream or in urine whose levels rise or fall during this time window.

Finding that antiangiogenesis drugs can normalize vasculature should not suggest that the original purpose for which they were developed is no longer valid. If a drug is sufficiently potent and specific to destroy enough tumor vasculature to starve the entire tumor and save a patient's life, then that would be a happy outcome for everyone. But the ability to use these drugs for vascular repair as well makes them valuable tools for attacking tumors in more than one way. In the longer term, this research can also benefit the many millions of people around the world suffering from other diseases caused by abnormal vasculature, such as age-related macular degeneration and atherosclerosis [see box on opposite page].

More than 30 years ago, when I first set out to understand the tortuous and dysfunctional blood vessels of tumors, I never imagined where that road would lead. Nor could I have pictured a day when a patient with a disease of abnormal blood vessels could walk into a clinic, have various biomarkers measured, then receive a tailored regimen of normalizing drugs to repair those vessels. But now that day looks closer than ever before.

**KEY CONCEPTS**

* Abnormal and dysfunctional blood vessels are a hallmark of solid tumors, one that contributes directly to malignant properties of a cancer as well as preventing treatments from reaching and attacking tumor cells.
* Normalizing tumor vessels allows cancer therapies to penetrate the mass and to function more effectively.
* Unexpectedly, drugs originally designed to destroy tumor blood vessels act to repair them for a time, opening a new avenue for cancer treatment as well as restoration of abnormal vasculature in other diseases. --The Editors

**MORE TO EXPLORE**

Normalization of the Tumor Vasculature: An Emerging Concept in Anti-Angiogenic Therapy. Rakesh K. Jain in Science, Vol. 307, pages 58-62; January 2005.

Lessons from Phase III Clinical Trials of Anti-VEGF Therapy for Cancer. Rakesh K. Jain, Dan G. Duda, Jeffrey W. Clark and Jay S. Loeffler in Nature Clinical Practice Oncology, Vol. 3, No. 1, pages 24-40; January 2006.

Angiogenesis in Brain Tumors. Rakesh K. Jain et al. in Nature Reviews Neuroscience, Vol. 8, pages 610-622; August 2007.

Antiangiogenic Therapy for Normalization of Atherosclerotic Plaque Vasculature: A Potential Strategy for Plaque Stabilization. Rakesh K. Jain et al. in Nature Clinical Practice Cardiovascular Medicine, Vol. 4, No. 9, pages 491-503; September 2007.

DIAGRAM: BRAIN TUMOR response to antiangiogenesis therapy reflects some effects of vascular normalization. MRI images of a patient's brain one day before beginning treatment and at subsequent intervals show a shrinking white area corresponding to the malignant tumor's location (top row). Another set of views highlights accumulated fluid in the same area receding (middle row). As tumor-generated edema diminishes, reduced compression of brain tissue is visible in the rebounding of fibrous white matter tracts, which are color-coded here by brain region {bottom row).

PHOTO (COLOR): HEALTHY VESSEL GROWTH AND MAINTENANCE: Endothelial cells form blood vessels in response to signals from molecules that promote and inhibit growth. Pericyte cells straddle vessels, and a basement membrane surrounds them; both provide support.

PHOTO (COLOR): BLOOD VESSELS in a normal mouse muscle's capillary bed (far left) and inside a mouse tumor differ distinctly. The tumor vessels branch erratically, vary in diameter along their lengths and are generally oversize -all features that contribute to irregular blood flow.

PHOTO (COLOR): TUMOR VESSELS cast in polymer from a surgically excised colon cancer form a dense crystalline mass representing a chaotic profusion of microvessels. In contrast, large gaps indicate areas of the tumor that lacked any blood supply.

PHOTO (COLOR): Abnormal blood vessels add to the havoc inside a tumor and prevent treatments from reaching cancer cells. Normalizing those vessels makes them into functional weapons that can be turned against the tumor.

PHOTO (COLOR)

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By Rakesh K. Jain

Rakesh K. Jain is Andrew Werk Cook Professor of Tumor Biology and director of the Edwin L. Steele Laboratory for Tumor Biology in the radiation oncology department of Massachusetts General Hospital and Harvard Medical School. His research incorporates biology, imaging, engineering and mathematics in the study of blood and lymphatic vessels and their tissue environment, as well as the adaptation of basic findings to patient treatment. He would especially like to acknowledge the National Cancer Institute for continuous support of his work since 1980 and more than 200 graduate students, postdoctoral fellows and collaborators worldwide who have shared his journey into the world of solid tumors. Jain also serves as an adviser to several pharmaceutical and biotechnology companies and is a member of both the National Academy of Engineering and the Institute of Medicine.

**ABNORMAL VESSELS MAKE TROUBLE**

**THE PROBLEM**

Malformed vasculature inside a tumor turns a bad situation worse (boxes). Flaws in the organization and functioning of blood vessels create barriers that prevent therapies from reaching tumor cells and foster an environment where those treatments are less effective. These unnatural internal conditions also contribute to malignant properties of the cancer itself.

**VESSEL ORGANIZATION**

* Oversize diameter and chaotic layout create irregular blood flow
* Absent or immature vessels make some tumor regions impenetrable

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**TUMOR MICROENVIRONMENT**

* Dysfunctional vessels produce conditions of low oxygen(hypoxia) and high acidity
* Radiation and certain chemotherapies that require oxygen to kill tumor cells are ineffective
* Immune cells that might attack cancer cells cannot function in an acidic environment and without oxygen Hypoxia causes changes in gene activity that promote tumor cell migration toward healthy tissues

**FLUID BUILDUP**

* Tumor tissue swells, causing painful symptoms
* Fluid pressure drives tumor-generated proteins and cells toward healthy tissues and into lymphatic vessels, increasing risk of metastasis

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PHOTO (COLOR)

PHOTO (COLOR)

**Vessel Repair: Beyond Cancer**

Hundreds of millions of people around the world suffer from noncancerous conditions that involve abnormal vasculature. Modifying blood vessel growth and function might become a key component of the therapeutic arsenal for those diseases as well, so drugs that normalize blood vessels have the potential to vastly impact human health.

Among the most widespread of problems in this category, for example, is atherosclerosis, an artery disease whose features include an accumulation of fatty plaques within the inner walls of blood vessels. Inside such plaques, inflammation-inducing blood cells and other detritus accumulate, gradually enlarging the lesion. New blood vessels sprout within this growing mass to feed it, much like a tumor. These new vessels also share many abnormal features with tumor vessels, such as leakiness and disorganization. In principle, therefore, applying antiangiogenic agents should normalize intraplaque vessels, stabilizing the lesions, halting their expansion and reducing their potential for rupture.

Eye diseases such as diabetic retinopathy and the so-called wet form of age-related macular degeneration (AMD) are also characterized by vascular abnormalities similar to those seen in tumors. A hallmark of wet AMD is, in fact, the leakiness of blood vessels in the retina at the back of the eye. As a result, blood oozes into surrounding tissue, causing partial or total vision loss. More than nine million Americans are currently affected. Not surprisingly, the greatest progress outside the realm of cancer treatment in using antiangiogenesis to repair vascular abnormalities has been in wet AMD. Two drugs, Lucentis and Macugen--both inhibitors of VEGF--have already been approved for treating the condition and most likely work by normalizing the leaky vessels.

These same normalization principles may also be useful for controlling conditions that cause fluid buildup (edema) and for tissue engineering and regenerative medicine, which require the creation and maintenance of normally functioning vasculature.--R.K.J.

PHOTO (COLOR): WET AMD affects the macula, a region of the retina at the back of the eye that appears slightly darkened in a healthy human eye (left) but can sprout excess blood vessels leading to macular degeneration. The abnormal vessels leak blood (right) into surrounding tissue, obscuring vision.

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