and propagates it along the narrow path needed for high acceleration. The JAERI group, however, claims to be seeing these phenomena, called self-focusing and self-channeling, at much lower energy levels. As evidence, they cite the shape of the fluorescence excited by the laser in the plasma, the spectrum of the laser light scattered out of the plasma, and direct measurements of the diameter of the laser beam, which they say remained small for several centimeters. But UCLA's Joshi and others say the evidence is not conclusive. "What is lacking is a measure of laser intensity in the channel," Joshi says.

The experimental observations are bolstered by computer simulations by JAERI physicists Yasuaki Kishimoto and James Koga. When the researchers assumed that the laser pulse itself ionized the gas, which is the approach used in most experiments, they found that self-channeling did not occur even at the theoretically predicted energy levels. However, if the gas were ionized ahead of the pulse, then self-channeling occurred well below the critical power that theory predicts. Koga speculates that this occurs in some experimental setups when a spike of energy from the laser pulse precedes the rest of the pulse and ionizes the gas.

His interpretation could explain why some groups, using laser pulses with different characteristics, have not detected self-channeling. But it leaves open the mechanism through which the self-focusing and self-channeling could be occurring. Says Koga, "We're wary about saying anything too strongly" about self-channeling. But Toshiki Tajima, a physicist at the University of Texas, Austin, who also does theoretical work at JAERI, says there is no other explanation for the results: "We may not understand the mechanism, but there has to be self-channeling."

The unexpected self-channeling laser isn't the only puzzle in the results, however. Wake-field acceleration is supposed to work only at a specific plasma density for any given laser pulse length, yet the JAERI group has seen electron acceleration over a broad range of plasma densities. They are convinced, however, that a wake field is responsible. In one set of experiments, the group varied the timing of the injection of the electrons relative to the laser pulse at different densities. Nakajima says the results—electrons injected too early or too late did not gain energy—support his contention.

Kwan Je Kim, a physicist at Lawrence Berkeley National Laboratory on sabbatical at Kyoto University, says questions about the results indicate that the whole area of wake-field acceleration is "a bit immature." But Tajima is more optimistic: "A year ago, we never thought the field would make such rapid progress." Theoretical explanations, he adds, will come hand in hand with additional data.

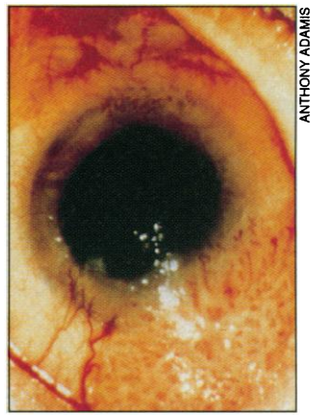
–Dennis Normile

CANCER RESEARCH

Designing Therapies That Target Tumor Blood Vessels

The word "cancer" conjures up images of a cohort of rampaging cells, burgeoning into life-threatening tumors that dispatch their metastatic offspring to ravage other parts of the body. Traditional cancer treatments have been based on attacking the rebel cells directly, by removing them surgically or attempting to destroy them with radiation or chemotherapy. But a new wave of potential cancer therapies aims to kill these hostile armies not by direct attack, but by shutting off their supply lines: the blood vessels through which tumors get the oxygen and nutrients they need to live and grow.

Work reported today advances this anticancer strategy further, giving a boost to two different means of cutting tumors' lifelines. The most common one aims to prevent tumors from forming the new blood vessels necessary to nurture their growth. To block the process, called angiogenesis, researchers have identified agents that interfere with the endothelial cells that build the new vessels, by preventing them from responding to growth factors or suppressing their ability to chew their way through surrounding tissues. The second approach seeks to block blood vessels that have already formed.



ANTHONY ADAMIS

Lifelines. A human eye cancer attracts new blood vessels.

These antiangiogenic and antivascular measures have already produced encouraging results on animal tumors, and today's reports add to the promise that has already launched a dozen or more candidate drugs toward the ultimate test in the clinic. In work described on page 547 of this issue, Philip Thorpe and his co-workers at the University of Texas Southwestern Medical Center in Dallas show that they can shrink or even eliminate tumors in mice by giving the animals agents that trigger blood clot formation in existing tumor-feeding vessels. And in today's issue of *Cell*, Judah Folkman and his colleagues at Harvard Medical School report their discovery of a factor called endostatin that is the most potent yet in a growing collection of molecules that block new blood vessel formation. Endostatin, Folkman's group reports, can shrink large tumors down to microscopic size in mice.

To see tumors shrink so dramatically under treatment is "outstanding ... better than my best hopes," says Noel Bouck of Northwestern University, who is also working on antiangiogenesis drugs. "If it just works for human tumors, it will be fabulous."

That, of course, is a very big "if," and one that applies to all the new strategies. As cancer researchers know only too well, many approaches that have looked promising in animals have died a quiet death after proving ineffective in humans. Still, antiangiogenic therapy may have unique strengths. For one, these drugs might avoid one of the main handicaps of conventional cancer therapies: the development of drug resistance, which ultimately leads to treatment failure.

"Cancers have a formidable ability to acquire resistance to any therapeutic modality we throw at them ... chemotherapy, radiation therapy, immunotherapy," says tumor biologist Bob Kerbel of the Sunnybrook Health Science Center at the University of Toronto. But the cells of a tumor's blood vessels—which are the target of the new therapies—are normal and thus less prone to mutate than cancer cells. As added bonuses, an effective vessel-targeting therapy should be useful for many types of cancer because all tumor-feeding blood vessels are essentially the same, and delivering a drug to the vessels should be much easier than getting it into all the cells of a solid tumor.

The idea of attacking a tumor's blood supply took some time to catch on. Back in the 1970s, when Folkman proposed that tumors have to induce new blood-vessel growth to obtain the nourishment they need, other researchers were skeptical. "The view of most scientists was that tumors didn't need blood-vessel growth at all, that they could grow with the supply that was there," Folkman says. Over the next decade, it became increasingly clear that Folkman was right, as his group showed that tumors contain newly formed blood vessels and secrete diffusible factors that cause those vessels to grow. In the early 1980s, Folkman's lab and others isolated several of those factors and showed that they trigger blood-vessel growth. They also identified the first antiangiogenic agents, platelet factor 4,

made by blood platelets, and fumagillin, a product of molds. Both showed ability to inhibit tumor growth in mice and have since entered clinical trials for cancer.

But even today, no one knows for sure how fumagillin and platelet factor 4 block angiogenesis, or whether they might have other unknown actions that account for their effects on tumors. Indeed, "compelling proof" that blocking angiogenesis could halt tumor growth didn't come until 1993, Folkman says, when Napoleone Ferrara's team at Genentech Inc. in South San Francisco showed that antibodies that arrest the activity of an angiogenic protein called vascular endothelial growth factor (VEGF) slow the growth of several types of tumors in mice.

After the Genentech discovery, several companies began developing drugs that block the action of VEGF or basic fibroblast growth factor, both of which stimulate angiogenesis by enhancing endothelial cell growth. Some of the drugs, which include antibodies to the growth factors as well as molecules that clog the growth factors' receptors on endothelial cells, have been shown to slow or stop the growth of tumors in mice; several are nearing clinical trials.

And recently, researchers in several labs uncovered another potential target for growth-factor inhibitors, a new family of receptors, called TIE receptors (for tyrosine kinase with immunoglobulin- and EGF-like domains) that exist almost exclusively on endothelial cells. George Yancopoulos and his colleagues at Regeneron Pharmaceuticals Inc. in Tarrytown, New York, have found a completely new family of naturally occurring molecules that they call angiopoietins. These either activate or inhibit the TIE receptors, which in turn influence endothelial cell growth. "We are working to move them forward" as potential therapies for diseases where angiogenesis needs to be either encouraged or inhibited, says Yancopoulos.

Other avenues. Blocking growth-factor stimulation of endothelial cells is just one way to stop blood-vessel growth. Indeed, some familiar drugs have as-yet unexplained antiangiogenic activity. The notorious tranquilizer thalidomide is enjoying a renaissance as a potential cancer therapy, thanks to the Folkman group's report in 1994 that it inhibits angiogenesis. How it does this isn't known, but its antiangiogenic properties may explain why limbs failed to form in some of the children born to mothers who took thalidomide as a morning sickness drug in the 1960s, says Folkman.

Other drugs that were already being tested for cancer therapy have also turned out to be angiogenesis inhibitors. These include the immune-signaling molecule interleukin-12 (IL-12), which was being developed as a cancer drug by Hoffmann-La Roche in Nutley, New Jersey, and the Genetics Institute in Cambridge, Massachusetts, because it activates killer T cells to attack tumors. Gary Truitt of Roche noticed that the tumors in IL-12–treated mice were pale and seemed to lack blood vessels, and he worked with Folkman's group to show that IL-12 blocks capillary growth. In at least one experimental system—mice with melanoma—it seems that the angiogenesis-inhibiting effects of the drug, rather than its immune-activating properties, may be what keep tumors at bay, says Maurice Gately, the IL-12 research leader at Roche.

SOME ANGIOGENESIS-INHIBITING DRUGS IN CLINICAL TRIALS

Drug (Sponsor)	Mechanism	Phase Trial (Cancer Types)
Marimastat (British Biotech)	Metalloproteinase inhibitor	III (pancreatic, lung, brain)
TNP470/AGM1470 (Takeda/Abbott)	Analog of fumagillin, an anti-angiogenic fungal product	III (breast, Kaposi's sarcoma, cervical)
Thalidomide (EntreMed)	Unknown	II (brain, breast, prostate)
CAI (NCI)	Ca channel blocker	I (wide range of cancers)
IL-12 (Roche, Genetics Institute)	Induces antiangiogenic protein IP-10	I (kidney)

Another important group of crossover drugs is the metalloproteinase inhibitors. These compounds block enzymes secreted by cancer cells that break down proteins of the extracellular matrix, enabling the cells to slip through the surrounding tissue and spread. Angiogenesis requires the same breakdown of the surrounding tissue to make way for new blood vessels, and the inhibitors have turned out to be "very potent" antiangiogenic compounds as well, says Henrik Rasmussen, vice president of research at British Biotech in Annapolis, Maryland, one of at least four companies developing metalloproteinase inhibitors for cancer.

In an early clinical trial, British Biotech's drug, Marimastat, seemed to slow disease progression and prolong life in patients with advanced colorectal, ovarian, and pancreatic cancer. But it is unclear, Rasmussen notes, how much of the drug's success depends on its ability to block blood-vessel growth rather than on its direct effects on the movement of cancer cells.

While all these drugs seem to slow or stop tumor growth, at least in animals, few researchers expected that they would actually shrink tumors that already had a blood supply. But newer results have surprised researchers and revised those expectations.

David Cheresh and his colleagues at the Scripps Research Institute in La Jolla, California, reported 2 years ago that they could halt tumor angiogenesis in chick embryos and mice by blocking the function of a molecule called integrin $\alpha_v\beta_3$, which is found on the surface of endothelial cells. Integrins interact with the proteins of the extracellular matrix and, in so doing, help the cells differentiate, migrate, and divide—just what they need to do to form new blood vessels.

When Cheresh's group treated animals with molecules that bind to and block the activity of integrin $\alpha_v\beta_3$, tumors in those animals not only didn't grow; in some cases they disappeared partly or completely. "This was the first indication that an angiogenesis inhibitor could have such a profound effect on the existence or proliferation of a tumor," Cheresh says. And it spurred the group to organize preliminary clinical trials of several integrin $\alpha_v\beta_3$–blocking drugs, which will soon begin in patients with advanced colon, lung, breast, and prostate cancer.

In retrospect, says Folkman, it's not surprising that blocking the formation of new blood vessels can shrink tumors. The entire capillary bed that feeds a tumor is continually remodeling itself, he says: Blood vessels "are being recruited, disappearing, and being recruited again. If you have a tumor that is even several grams, for it to stay at that size, it is always recruiting new vessels. When you stop the angiogenesis, you begin to get capillary dropout." And the tumor, starved of blood, begins to shrink.

Potent inhibitors. Perhaps the most dramatic tumor-shrinking drugs have come from an unlikely source: tumors themselves. Cancer researchers have known for many years that removal of a primary tumor sometimes causes a burst of growth in distant metastases, suggesting that the primary tumor had been making a substance that kept the metastases in check. In the late 1980s, Northwestern's Bouck and her colleagues made a discovery that hinted at what might be going on. Some tumor cells, they found, although able to induce angiogenesis in the tissue around them, for some unknown reason also make angiogenesis inhibitors. Apparently, the balance of inducers to inhibitors is favorable for vessel growth in the vicinity of the tumor, but at sites distant from the tumor, the inhibitors seem to prevail.

One inhibitor Bouck's team found to be made by some tumor cells is thrombospondin, a protein also found in platelets and in the

extracellular protein matrix. It "makes endothelial cells unable to respond to any [angiogenesis] inducer we have tried," says Bouck. How it does that remains a mystery, although Scripps's Cheresh notes that thrombospondin in the extracellular matrix binds to cell surface receptors including integrin $\alpha_v\beta_3$. That interaction, he suggests, may interfere with the endothelial cells' ability to form new blood vessels.

Bouck's team has since found that thrombospondin blocks the metastasis of melanoma cells into the lungs of mice. And Pat Steeg and her colleagues at the National Cancer Institute have shown that breast cancer cells engineered to make high levels of thrombospondin lose some of their ability to promote angiogenesis and to metastasize.

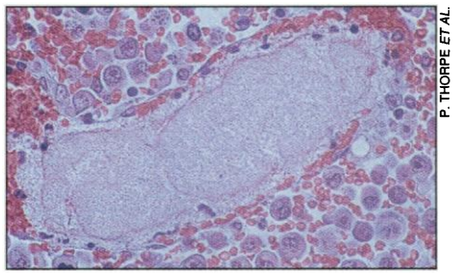
Two years after Bouck's 1989 finding, Folkman and then-postdoc Michael O'Reilly identified and then eventually purified another angiogenesis inhibitor, which was produced in mice by a metastasis-limiting tumor called Lewis lung carcinoma. Last June, Folkman's team reported that the inhibitor, angiostatin, not only stopped experimental tumor growth in mice, but shrank the tumors dramatically. Tumors that had been allowed to grow to 400 milligrams—about 2% of the weight of the mouse—shrank to microscopic size with angiostatin treatment.

And in today's issue of *Cell*, the Folkman team reports purifying yet another antiangiogenic factor, from a different metastasis-limiting tumor. This protein, which they call endostatin, is even more potent. It shrank a wide variety of tumors in mice to microscopic sizes, keeping primary tumors and metastases in check as long as the mice received the drug.

The means by which angiostatin and endostatin block angiogenesis is unknown, says Folkman. But Cheresh suspects that, like metalloproteinase inhibitors, they interfere with the remodeling of the extracellular matrix that has to happen for new blood vessels to grow. He notes that both proteins are pieces of larger proteins that play key roles in the extracellular matrix. Angiostatin comes from plasminogen, which is normally cleaved into the matrix-digesting protein plasmin, and endostatin comes from collagen. Cheresh suggests that angiostatin and endostatin, as free-floating fragments of these proteins, may clog the active sites of the enzymes that chop up plasminogen and collagen, and so prevent endothelial cells from forming new blood vessels.

No one knows whether the compounds will produce such dramatic results in humans, and clinical trials are still a few years off, says Folkman. Toronto's Kerbel cautions that it may be unrealistic to expect tumor shrinkage in humans, because human tumors will have been growing for much longer than the tumors treated in mice. "When you have tumors that may have been there for 5 to 10 years, the nature of the vasculature might be quite different," he says, and less vulnerable to a blockade of angiogenesis.

Still, the mouse results suggest that the new angiogenesis inhibitors will not suffer from the bane of conventional cancer chemotherapies: the development of resistance. When Folkman's team took their mice off endostatin, the tiny tumors began to grow back. But they remained responsive, shrinking again when the drug was readministered. As of 18 November, when Folkman reported these unpublished results during a conference at the Institute of Human Virology in Baltimore, the mice had been through six cycles of endostatin administration and withdrawal; each time the tumors grew to hundreds of milligrams and shrank back to microscopic size. That result is "unprecedented," says Kerbel. "That would just never happen with chemotherapy." If it's an indication of how endostatin would work in humans, says Folkman, it would mean treatment could be restarted if metastases were to grow back.



P. THORPE *ET AL.*

Blocked. New therapy induces a blood vessel–filling clot in a mouse's tumor.

They probably would, because tumors smaller than 1 to 2 millimeters don't need an induced blood supply but can live on existing blood vessels. In practical terms, that might mean that a patient would have to take such inhibitors continually to hold metastases in check, unless they could be eliminated by combining the treatment with conventional chemotherapy. But some clinicians worry about possible complications of long-term antiangiogenesis treatment, because blood-vessel growth is critical to processes such as wound healing.

Well-placed clots. Despite all the attention that antiangiogenesis therapy is getting, it isn't the only means of attacking a tumor's blood supply. Thorpe and his team at the University of Texas Southwestern Medical Center report in this issue that they have had success with what might be called an antivascular approach. Their goal was to cause blood clots specifically in the blood vessels feeding a tumor and so starve the tumor of blood and oxygen.

Thorpe's team injected the mice with cancer cells engineered to make interferon γ, which induces nearby endothelial cells to make class II antigen, a protein they normally don't make. The researchers then took antibodies to class II antigen and linked them to a shortened version of tissue factor (TF), a protein that normally sits outside blood vessels and triggers blood clotting at injury sites. Because the TF they used is missing a membrane-attachment region, it is powerless to form clots. The team reasoned that by linking it to the antibody, they could direct it to the class II antigen on the walls of the tumor blood vessels, thus bringing the TF back in contact with the vessel walls' membranes and causing clots to form specifically in those vessels.

The experiment "worked like a charm," says Thorpe. The antibody-TF complex "homed in on the tumor vessels as expected and caused rapid, selective, and complete thrombosis of the tumor's blood supply." Indeed, clots formed in the tumor vessels within minutes of the injection, and the tumors were largely dead within 48 hours. In 38% of the mice, the tumors disappeared completely, while in others a rim of cells around the outside of the tumor, which don't depend on the tumor's blood vessels, survived. But those cells, Thorpe says, are rapidly dividing and are "sensitive to conventional cancer chemotherapeutic drugs."

Thorpe's approach "has promise," says Robert Tressler, a tumor biologist developing anticancer drugs at Chiron Corp. in Emeryville, California. But he notes that before it can be used in humans, researchers will have to make sure that the drug doesn't cause clotting in nontumor vessels. Thorpe is optimistic that he can do that, because blood vessels around tumors are much more disposed toward blood clotting than normal vessels are. Even when his team deliberately mistargeted the truncated TF to a highly expressed marker on normal vessels, he says, they saw virtually no clotting.

Thorpe points out that the tumors given to the mice had been engineered to induce the class II marker on the tumor-associated blood vessels, something that could not be done in human cancer patients. But, he says, there are several candidate proteins on newly formed blood vessels that could be used as targets in humans. "This isn't pie in the sky," Thorpe says. "There are very real opportunities for use of these agents in humans." He hopes to have the approach in clinical trials within 2 years.

Whether antiangiogenic and antivascular therapies turn out to be pie in the sky or a realistic new approach for cancer rests on the results of the host of clinical trials planned for the next few years. But at this stage at least, a lot of researchers and companies are placing their hopes on this new strategy for cutting the lifeline to tumors.

–Marcia Barinaga