

MEDICINE

An Indirect Way to Tame Cancer

Cells and a stiff material called the matrix can squeeze blood vessels in tumors and thus block delivery of cancer-fighting drugs to malignant cells.

Now researchers may have a way to reopen vessels and restore the drugs' power

By Rakesh K. Jain

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OR NEARLY 40 YEARS, I HAVE BEEN WORKING ON FIGHTING CANCER FROM AN unusual angle. Trained initially as an engineer, I see tumors in part as a physics challenge and ask: How do their structural features promote their growth and keep cancer-fighting drugs from working effectively?

More than two decades ago, for instance, my co-workers and I, then at Carnegie Mellon University, revealed that structural abnormalities in tumor blood vessels interfere with drug delivery to malignant cells in a mass. These vessels tend to be overly twisty and porous, and the porosity leads fluid and drugs in the blood to leak out as blood enters a mass. The fluid then exerts an outward pressure that causes it and the drug molecules to ooze out of the tumor into the surrounding tissue. We later showed that reducing the leakiness could also lower this so-called interstitial fluid pressure and improve drug distribution in the tumor, thereby enhancing responses to various treatments meant to attack cancer cells.

More recently, our research has demonstrated that fluid pressure is not the only troubling physical force at work. Tumors are a mash-up of malignant cells, nonmalignant cells, and blood and

lymph vessels, all embedded in a fibrous material known as the extracellular matrix. The solids—the matrix and cells—can squash the lymph and blood vessels. This compression, known to physicists and engineers as solid stress, may reduce or halt blood flow to many parts of the tumor, which can, in turn, reduce drug delivery and also set up conditions that foster cancer progression. Meanwhile the matrix—which is abnormally stiff in tumors and is more abundant in some cancers than in others—can directly impede the dissemination of anticancer drugs throughout a mass.

Knowing all the worrisome roles played by the tumor matrix, my colleagues and I have lately been searching for ways to diminish it. We have now found an approach that appeals to us in part because it relies on a class of medicines already known to be safe: certain drugs prescribed widely for high blood pres-

IN BRIEF

In a tumor, cells and a material called the matrix can squeeze blood vessels shut, preventing them from delivering anticancer drugs to many parts of the mass. The matrix can also directly retard dispersion of

cancer-fighting agents through a tumor. **Squashing of blood vessels** can, moreover, deprive tumors of oxygen, an effect that can increase the aggressive behavior of cancer cells.

Evidence in mice indicates that depleting the matrix with a drug already used to control blood pressure can improve the perfusion of anticancer drugs in a tumor and improve survival rates.

This drug is now being tested in humans, and efforts are under way to find an agent that will deplete the matrix more effectively without lowering blood pressure too much.

sure. Human studies are under way to test such treatment in a type of pancreatic cancer that is one of the most matrix-rich and hardest-to-treat malignancies.

Of course, we cannot promise that matrix-depleting drugs will prove revolutionary. Cancer is actually many different diseases, all of them wily. But if the agents work as we hope, they could become a powerful new ally in the fight to prolong the lives of people stricken by cancers that too often defy eradication.

UNDER THE HOOD

I BEGAN THINKING ABOUT interfering with the matrix after I learned that the compression of blood and lymph vessels in tumors causes a startling array of troubling effects. For instance, lymph vessels normally remove excess fluids from tumors and other tissues. When lymph vessels inside a tumor get squeezed shut, they cannot drain liquid that has leaked from the tumor blood vessels, and so fluid pressure increases. Meanwhile the compression reduces the already compromised ability of blood vessels to distribute blood—and thus oxygen, tumor-fighting immune cells and anticancer drugs—throughout a malignancy and leaves many areas deprived of oxygen.

This oxygen scarcity, or hypoxia, might sound like it would be a good thing, blocking a tumor's ability to grow. In reality, however, it can be particularly nefarious. Oxygen deprivation in a tumor can spur malignant and even normal cells to secrete proteins that suppress the activity of tumor-fighting immune cells. One such protein, called vascular endothelial growth factor, or VEGF, also increases the leakiness of blood vessels, thereby further reducing blood flow in tumors and adding to the fluid pressure. Hypoxia, moreover, converts some immune cells from cancer fighters to cancer accomplices.

That is not all. Hypoxia favors survival of more malignant cells (those best able to invade tissues and spread) over less malignant ones, because the less dangerous cells tend to commit suicide when oxygen is scarce. What is worse, a dearth of oxygen can increase the invasive tendencies of cancer cells by, for example, inducing them to make proteins that help them travel away from the original mass. And oxygen deprivation undermines the functioning of many anticancer drugs.

The matrix also causes trouble in ways that do not involve oxygen deprivation and reduced drug delivery. My colleagues and I have recently discovered that mechanical compression can reprogram some cancer cells to become “leaders” that es-

entially start marching into nearby tissues and inducing other cells to follow. What is more, in a vicious cycle, compression and hypoxia can each prod dedicated matrix-producing cells, such as fibroblasts, to up their activity and can spur certain cancer cells to secrete matrix components even though nonmalignant versions of those cells do not participate in matrix production.

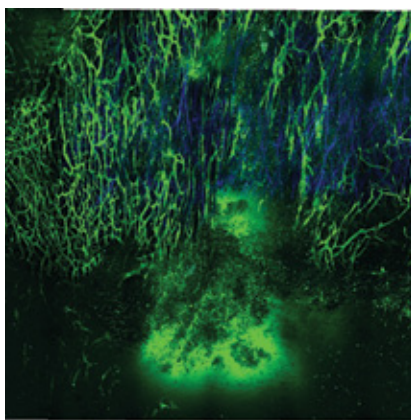
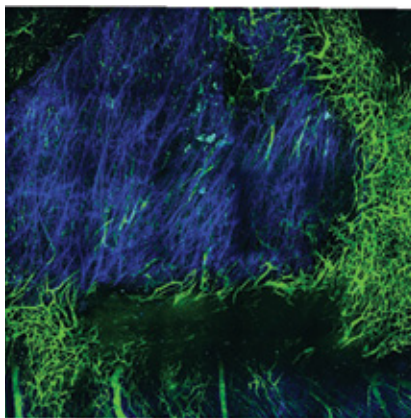
To be sure, my group understood the consequences of hypoxia even before we began work on the matrix, and we made an early priority of finding ways to reduce it. We first proposed about 13 years ago that “normalizing” the vessels—making them less twisted and leaky—would improve blood perfusion through the tumor and reduce fluid pressure, thereby reducing hypoxia and its effects and facilitating delivery of drugs and immune

cells. We have now provided compelling evidence supporting this hypothesis in both animals and humans. Indeed, we have shown that restoring some normalcy to blood vessels—which can be achieved with inhibitors of new blood vessel formation (so-called antiangiogenic drugs)—is accompanied by increased blood flow and oxygen delivery through brain tumors and, crucially, can increase some patients' survival time. This mechanism can also explain increased survival in patients with colon, lung and kidney cancer who received the antiangiogenic drug bevacizumab together with chemotherapy (drugs that kill the rapidly dividing cancer cells) or immunotherapy (treatments that are meant to enhance the body's immune response against tumors).

Researchers continue to optimize the approach, but it will never be enough on its own because fluid pressure is not what compresses blood and lymph vessels in tumors; the matrix and cells in a tumor do that. (When fluid pressure increases around leaky vessels, fluid seeps back through the pores, rather than collapsing the vessels [see box on page 52].) Antiangiogenic drugs cannot open vessels that are clamped shut by the matrix and cells. That is where our work on diminishing the matrix and thus alleviating the solid, compressive stress comes in.

Before searching for drugs that might deplete the matrix, we first wanted a better idea of how tumors differ in their abundance of the material and in how much stress the solid components exert. We found that cancers vary in this respect, although a look at many human tumor masses under the microscope told us that most harbor some collapsed vessels.

The extent of collapse depends in part on the stage of tumor progression and the location. Being in a confined space, for



MATRIX (blue) in a mouse tumor decreased (*top to bottom*), and blood flow increased (*green at center*), in response to a matrix-depleting agent. In other animal tests, such changes have improved the efficacy of anticancer drugs.

instance, will increase compressive stress and the number of vessels that are partially or totally collapsed. The kind of tumor plays a role as well. The most common type of pancreatic cancer (pancreatic ductal adenocarcinoma), for instance, usually has relatively few cancer cells, which constitute less than 5 percent of the mass, and contains a lot of matrix and fibroblasts. Other cancers, though (such as medulloblastoma, the most common pediatric brain cancer), typically have relatively little matrix. Additional work has shown that, in general, tumors with a high ratio of matrix and fibroblasts to cancer cells—termed desmoplastic—tend to be least responsive to drug therapy, suggesting that reducing the amount of matrix in such masses might improve the odds that the drugs will reach their targets and thereby work better than usual.

FIRST TESTS

MY GROUP'S INITIAL EFFORTS at finding agents able to minimize the matrix were aided by a chance discovery. The matrix consists of protein fibers (mainly composed of collagen) and of gel-like components, such as hyaluronan. At one time, investigators thought that the gel-like molecules played a bigger role in interfering with drug delivery through tumors than collagen did. But in 2000 we found, to our surprise, that the stiffness of the tissue, which is a function of how much collagen it contains, is more important. We also found that breaking up the fibers with a collagen-degrading enzyme—collagenase—greatly increased distribution of a 150-nanometer particle meant to stand in for a drug, even in the most penetration-resistant tumors. (We tested particles of that size as proxies for the nanoscale medicines that are increasingly being studied as targeted cancer cell killers but that so far have seen only modest success.) Following up on this discovery, we showed that tumors in mice shrank more when we injected the masses with a collagenase along with 150-nanometer viral particles meant to kill cancer cells than when we injected the viral particles without collagenase.

As interesting as that result was, we knew that administering an enzyme that would degrade collagen throughout the body would be problematic in humans: collagen gives structure to our bones and tissues. We needed a safer drug that worked in tumors without producing widespread effects. But what?

We soon considered a hormone called relaxin, which women produce during pregnancy. Relaxin inhibits collagen synthesis and enhances its breakdown, and mothers-to-be make a lot of it with no ill effects. We therefore wondered whether it might be used to decrease collagen in tumors.

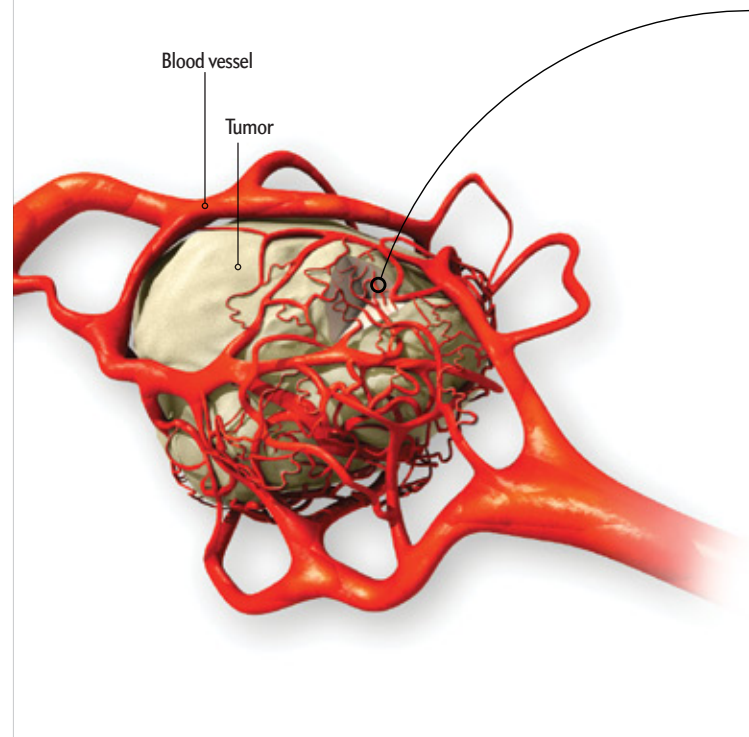
In 2002 we treated tumor-bearing mice with relaxin for two weeks. Sure enough, it reorganized collagen, making it more porous and improved dispersion through the tumors of large molecules that we used as stand-ins for drugs. Others confirmed our findings with different tumors. But then we learned of research indicating that that relaxin could enhance the progression of some tumors, such as those in the prostate. Given the disparity of findings and the risks involved, we knew we could never test such a drug for cancer therapy in humans.

BETTER LUCK THIS TIME

DISAPPOINTED, WE STARTED to search for other agents. We decided to focus on attacking one key player involved in collagen syn-

Forces to Be Reckoned With

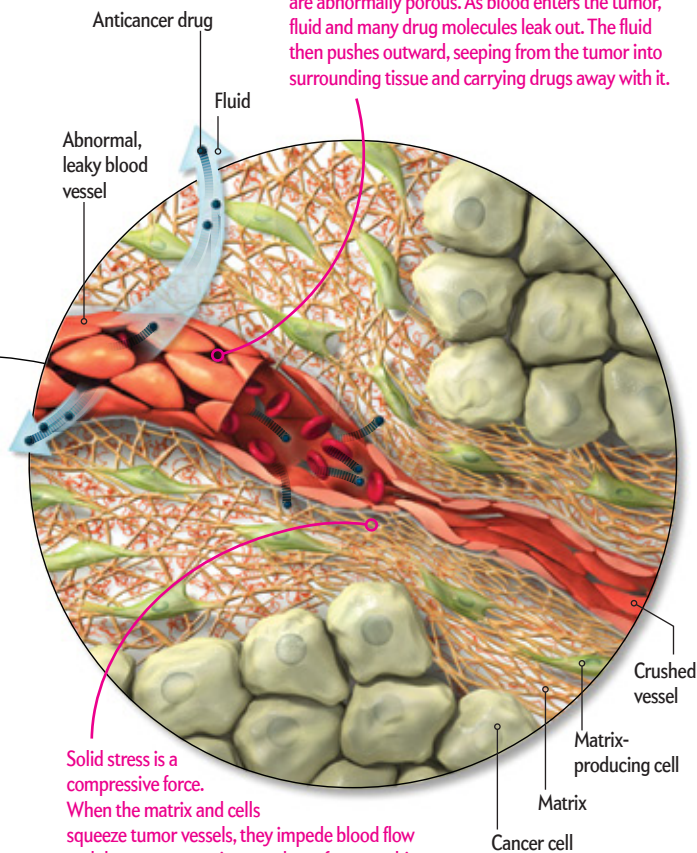
In theory, anticancer drugs delivered to a tumor via the bloodstream would reach everywhere in the mass and attack all cancer cells within it. But forces exerted by fluid and solids in the tumor—including those known as fluid pressure and solid stress—can be high enough to block the drugs from reaching many of the cells they are meant to kill (*right*). Cancer specialists already have access to agents that can help reduce fluid pressure. And now, as the author explains in this article, researchers think they may also have a way to reduce the solid stress, which is exerted by the matrix and cells (*far right*).



thesis—a protein named transforming growth factor beta. We found a possible way to do that when we realized that a class of medicines commonly used to treat hypertension (high blood pressure) not only lowers blood pressure but, usefully for us, has a second effect in the body: inhibiting the growth factor's activity. What is more, these widely prescribed agents—which lower blood pressure by inhibiting a hormone called angiotensin II—also impair the function of a second molecule, involved in stabilizing collagen. We knew that various angiotensin inhibitors, including one called losartan, reduce levels of several forms of collagen in laboratory animals that have excessive amounts of extracellular matrix and can reverse such excess in the kidneys and hearts of hypertensive patients. But we could not find any published research addressing the effect of these

The Barriers

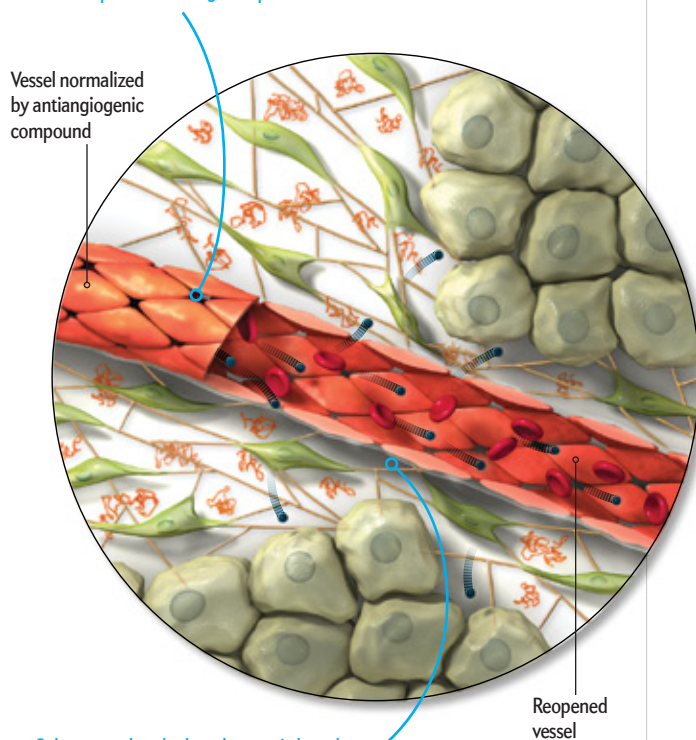
Fluid pressure is often high because tumor vessels are abnormally porous. As blood enters the tumor, fluid and many drug molecules leak out. The fluid then pushes outward, seeping from the tumor into surrounding tissue and carrying drugs away with it.



Solid stress is a compressive force. When the matrix and cells squeeze tumor vessels, they impede blood flow and thus prevent anticancer drugs from reaching malignant cells beyond the crushed region. At the same time, the matrix can trap drug molecules, further limiting their dispersal in a mass. Crushing of blood vessels can also starve tumors of oxygen, an effect that can promote a cancer's spread and hinder immune attacks on it (not shown).

Solutions

"Antiangiogenic" medicines can reduce fluid pressure and have been used in cancer care for about 10 years. They are best known for inhibiting the formation of new tumor blood vessels. But they can also "normalize" blood vessels, making them less leaky, so that less fluid seeps out and drugs can penetrate farther into a tumor.



Substances that deplete the matrix have been shown in mice to reduce solid stress and thus enhance tumor blood flow and the dispersal of anticancer drugs. These matrix-depleting substances include a common blood pressure medicine that is now being tested in patients with pancreatic cancer to see if it will improve the effectiveness of anticancer drugs.

drugs on collagen levels or on compressive stress in tumors.

To see if angiotensin blockers might diminish the matrix in tumors and thus improve the distribution of drugs that attack malignant cells, we gave losartan for two weeks to mice bearing four different types of matrix-rich tumors: pancreatic ductal adenocarcinoma, breast cancer, the skin cancer melanoma and cancer arising from connective tissue (sarcoma). We saw two encouraging effects. Collagen decreased in the tumors, and the 100-nanometer particles we delivered as proxies for cancer cell-killing drugs suffused tumors more fully than usual. We concluded that better penetration by the proxies occurred because of reductions in the collagen content. Follow-up studies in rodents, published in 2011, proved the case with actual nanomedicines: the FDA-approved drug Doxil (about 100 nanometers in diameter)

and viral particles (about 150 nanometers) that kill cancer cells.

During the course of our research, we also discovered that the higher the losartan dose, the greater the decrease in collagen. Such dose dependence is a good sign that a drug being tested accounts for an observed effect. The finding implied as well that doses of losartan higher than those we tested earlier might decrease collagen so much that the blood vessels in even matrix-rich tumors would open up enough to allow anticancer drugs to flow readily through tumor vessels and reach areas of the masses that formerly received no blood. Indeed, in mice, doubling the dose depleted collagen in matrix-rich breast and pancreatic tumors, opened up blood vessels and improved the delivery and efficacy not just of nanoscale drugs but also of standard chemotherapies used to treat these cancers.

We then wondered whether high-enough doses of losartan or other antihypertensives would increase the effectiveness of conventional chemotherapy and nanomedicines in humans with cancer. We do not have a definitive answer yet, but we have reason to be encouraged. Reviews of past studies involving cancer patients who also had hypertension, and thus received both cancer therapy and blood pressure medicines, imply that certain antihypertensive agents do seem to improve outcomes somewhat. One analysis of past research indicated, for example, that in combination with the anticancer drug gemcitabine, inhibitors of angiotensin or of certain related enzymes increased overall survival in pancreatic ductal adenocarcinoma patients by about six months relative to the survival achieved with chemotherapy alone.

Of course, retrospective studies have their limitations, but the data in cancer patients who were treated for hypertension are consistent with our findings in mice and provide a basis for testing angiotensin blockers as matrix-depleting agents in humans. Accordingly, a group at Massachusetts General Hospital

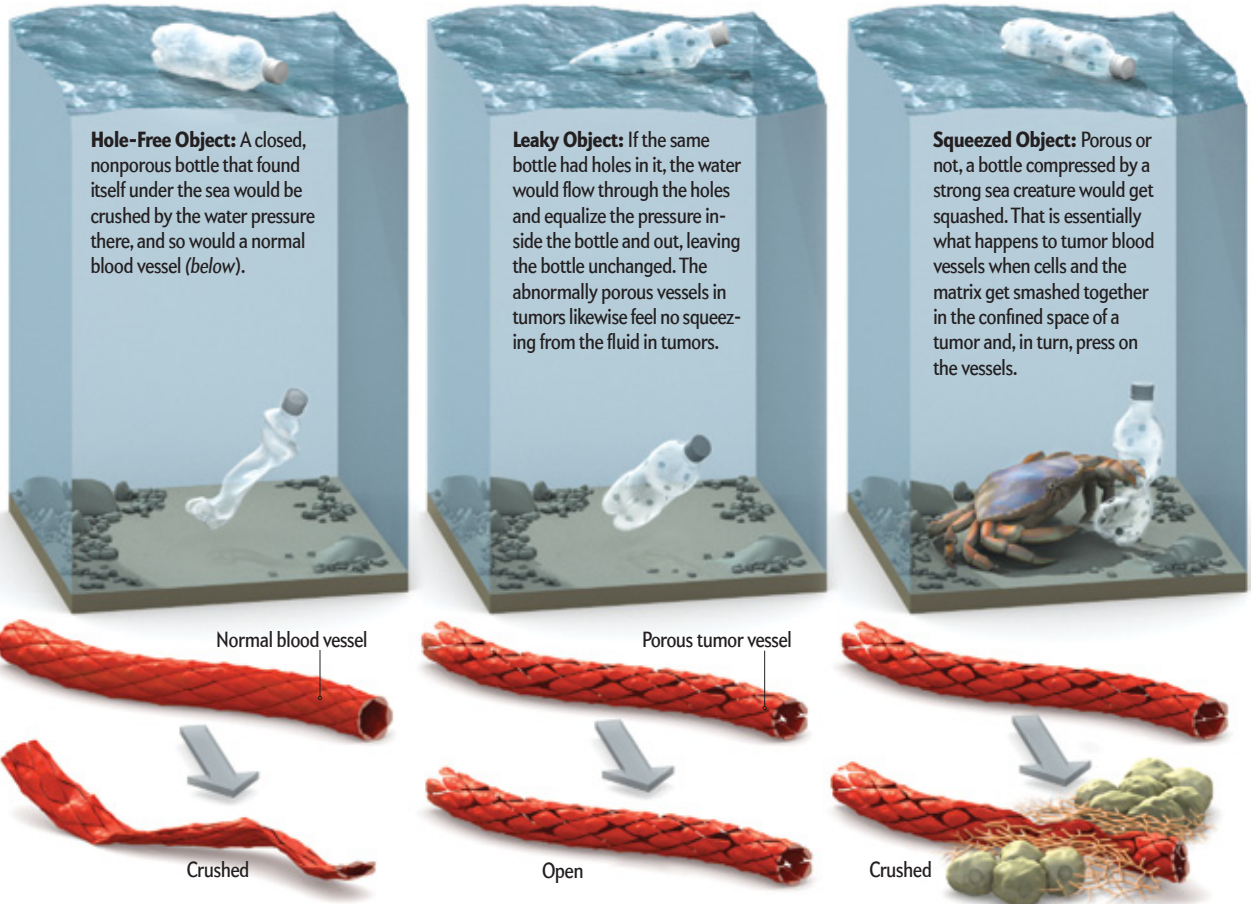
has initiated a trial of losartan with standard chemotherapy in patients with pancreatic ductal adenocarcinoma, which has a five-year survival rate of less than 6 percent. (I am not involved in this trial.) The results could be available in a couple of years. In the future, if all goes well, I can imagine improving patient care by delivering a combination of therapies that would include vessel-normalizing drugs (such as VEGF blockers), matrix-depleting agents and targeted cancer cell killers.

As is true of most medicines, antihypertensive drugs have some drawbacks. They cannot be given to people who have certain kidney diseases or who have low blood pressure. Even for patients with normal blood pressure, the doses need to be carefully monitored to avoid severe drops in blood pressure. These problems might be addressed by finding ways to alter angiotensin-blocking agents so that they retain the ability to diminish the matrix but do not also lower blood pressure. My colleagues and I are pursuing that goal. Still, tumors tend to develop resistance to most medicines. Whether they will show resistance to losartan or other angiotensin blockers is not known.

TUMOR PHYSICS 101

Why Only Solids Squeeze Tumor Vessels

People are often surprised to learn that only the matrix and cells in tumors squeeze blood vessels shut; the fluid buildup does not play a role in that effect. The author likes to explain the logic by analogy to a plastic soft-drink bottle dropped into the ocean.



SOURCE: LANCELETTI, EDWIN L. STEELE LABORATORY FOR TUMOR BIOLOGY, HARVARD MEDICAL SCHOOL AND MASSACHUSETTS GENERAL HOSPITAL

To improve treatment, researchers need to not only gain a better understanding of cancer's genetic underpinnings but also consider the physical forces in tumors.

ALTERNATIVES

WHAT ABOUT PATIENTS who cannot take antihypertensives or whose tumors do not respond well or persistently to them? Specifically targeting the nonfibrous, hyaluronan molecules in tumors might be another way to attack the matrix. Hyaluronan is abundant in about 25 percent of human tumors, such as pancreatic ductal adenocarcinomas and breast, colon and prostate cancers. We have recently shown that an enzyme that depletes it, a hyaluronidase, can indeed reduce solid stress in tumors grown in mice. We have shown as well that losartan can reduce hyaluronan in tumors. Others have demonstrated that a hyaluronidase can decompress blood vessels. Based on the latter studies, a formulation of the enzyme (called PEGPH20) is now in a clinical trial for pancreatic cancer. We and other groups have also had some success in the lab with certain other drugs known to act in part by affecting the matrix.

To perfect matrix-depleting therapy, researchers need ways to measure the response of the matrix to various test agents. Do substances that disrupt the tumor matrix actually reduce mechanical compression? Which ones are most effective? And does the degree of reduction make a difference to the success of more traditional cancer drugs delivered at the same time? Progress is being made on this front as well. A new imaging method, known as second-harmonic generation, should help investigators see and measure collagen in tumors. In addition, my colleagues and I have found a relatively simple way to gauge compressive stress in a tumor: When a tumor is cut in half, the halves spontaneously swell. Measuring the swelling and applying mathematical formulas we developed reveals the amount of stress the interior was under.

I am sometimes asked if depleting the matrix would make it easier for cancer cells to metastasize—to move through the matrix into blood and lymph vessels and then out into other parts of the body. Similarly, people wonder if digesting the matrix or otherwise opening vessels and improving blood flow through a tumor would help tumor cells escape into the circulation or promote tumor growth by delivering more nutrients to cancer cells, or both. These concerns need further study, but several observations suggest that therapies able to ease compressive stress and otherwise normalize vessels would *not* foster tumor growth and metastasis. Why? On one hand, it is true that nutrients would reach tumor cells, which would also be freer to move around; on the

other hand, the oxygen deprivation that promotes tumor progression, impairs immune responsiveness and lowers the efficacy of many therapies would be reduced. Moreover, greater quantities of delivered drugs and potentially more immune cells would be fanning out through the tumor and in the normalized, more open blood vessels to counteract any tumor-friendly effects of the therapy. Ongoing animal and human studies will show which of these effects are most powerful.

FOLLOW THE LOGIC

BACK WHEN MY COLLEAGUES and I first started considering angiotensin blockers for combating cancer, others we consulted discouraged us from pursuing this line of work. Because the agents *reduce* blood pressure, the thinking went, they would cause tumor blood flow to go down, not up. Furthermore, studies involving delivery of angiotensin—which, in contrast to angiotensin blockade, *elevates* blood pressure—found increased tumor blood flow in many studies of mice and humans. But that work did not consider the compressive effects of the matrix, and a trial assessing the effectiveness of angiotensin therapy for treating cancer in patients failed. A few years later we explained the failure: the drug enhanced blood flow only transiently, presumably because compressive stress quickly closed off the affected vessels.

As cancer researchers look to the future, we need to not only gain a better understanding of the genetic and cellular underpinnings of cancer but also consider the consequences of physical forces in tumors. We need to exploit all this knowledge to fully discern the laws that govern tumor progression and learn how to improve cancer detection and treatment. Solid tumors exploit physical forces for their survival. It is time for us to exploit our own knowledge of physics to fight back. ■

Disclosure of commercial ties: *Rakesh K. Jain co-founded, has equity in, and sits on the board of directors of XTuit, a company that is developing anticancer therapies. XTuit and Massachusetts General Hospital have applied for drug patents based on the work summarized in this article. Jain also receives grants and consulting fees from and advises several other companies involved in cancer research.*

MORE TO EXPLORE

Causes, Consequences and Remedies for Growth-Induced Solid Stress in Murine and Human Tumors. Triantafyllos Stylianopoulos et al. in *Proceedings of the National Academy of Sciences USA*, Vol. 109, No. 38, pages 15101–15108; September 18, 2012. www.pnas.org/content/early/2012/08/28/1213353109.full.pdf

Normalizing Tumor Microenvironment to Treat Cancer: Bench to Bedside to Biomarkers. R. K. Jain in *Journal of Clinical Oncology*, Vol. 31, No. 31, pages 2205–2218; June 10, 2013.

Angiotensin Inhibition Enhances Drug Delivery and Potentiates Chemotherapy by Decompressing Tumor Blood Vessels. Vikash P. Chauhan et al. in *Nature Communications*, Vol. 4, Article No. 2516; October 1, 2013. www.nature.com/ncomms/2013/131001/ncomms3516/pdf/ncomms3516.pdf

Find more on the matrix-depleting clinical trial being carried out by cancer specialists at Massachusetts General Hospital: <http://clinicaltrials.gov/show/NCT01821729>

Rakesh K. Jain's laboratory: <http://steele.mgh.harvard.edu>

FROM OUR ARCHIVES

Barriers to Drug Delivery in Solid Tumors. Rakesh K. Jain; July 1994.

Vessels of Life or Death. Rakesh K. Jain and Peter F. Carmeliet; December 2001.

Taming Vessels to Treat Cancer. Rakesh K. Jain; July 2008.