New Answers for CANCER

Cutting-edge drugs and research are helping solve the puzzle

Cancer Resource Guide
Where to turn for help

Stem Cells
The real culprits?

The Cancer Genome
Mapping out an attack plan
In This Issue

NEARLY 40 YEARS since we declared war on cancer, how goes the campaign against this intractable and ancient adversary? As you will learn in this special edition, our enemy intelligence has improved over the years, enabling us to get a better bead on where the trouble begins. And we have developed stronger weapons, to more precisely pursue and annihilate diseased tissue.

Finding Enemy Forces. Cancer’s origins are multifaceted, a combination of an individual’s genetic factors and influences from the surrounding environment and his or her personal history and lifestyle. Even stem cells—which, in other contexts, offer promise for the treatment of a variety of ailments—could be to blame. To learn more, turn to page 40 for “Stem Cells: The Real Culprits in Cancer?” by Michael F. Clarke and Michael W. Becker. And in “Mapping the Cancer Genome,” on page 22, Francis S. Collins and Anna D. Barker explain how such a tool will help us chart a course across the landscape of human malignancies.

Destroying the Targets. While scientists are grappling to gain a better understanding of cancer’s complex beginnings, they also have improved ways of stalling the advance of the disease. In “Taming Vessels to Treat Cancer,” on page 64, for instance, Rakesh K. Jain describes how calming the chaos in tumors’ blood vessels could facilitate attacking them. Francisco J. Esteva and Gabriel N. Hortobagyi explain how we are also “Gaining Ground on Breast Cancer” with targeted therapies, beginning on page 88.

Hope in the Trenches. Patients with cancer, empowered by expanding informational resources and the change, more open attitudes of doctors, are living longer and better than ever today, as Lisa Stein writes in “Living with Cancer”; see page 6. Although medicine clearly has much work to do, with advances occurring rapidly, we are on the path to managing this chronic disease.
Letter from the Editor

Living with Cancer
by Lisa Stein
Keep up your spirits and tap available resources to make the disease manageable.

Evolved for Cancer?
by Carl Zimmer
Natural selection lacks the power to erase cancer from our species and, some scientists argue, may even have provided tools that help tumors grow.

Mapping the Cancer Genome
by Francis S. Collins and Anna D. Barker
Pinpointing the genes involved in cancer will help chart a new course across the complex landscape of human malignancies.

Untangling the Roots of Cancer
by W. Wayt Gibbs
Recent evidence challenges long-held theories of how cells turn malignant—and suggests new ways to stop tumors before they spread.

Stem Cells: The Real Culprits in Cancer?
by Michael F. Clarke and Michael W. Becker
A dark side of stem cells—their potential to turn malignant—is at the root of a handful of cancers and may be the cause of many more. Eliminating the disease could depend on tracking down and destroying these elusive killer cells.

A Malignant Flame
by Gary Stix
Understanding chronic inflammation, which contributes to heart disease, Alzheimer’s and a variety of other ailments, may be a key to unlocking the mysteries of cancer.

The Long Arm of the Immune System
by Jacques Banchereau
Dendritic cells catch invaders and tell the immune system when and how to respond. Vaccines depend on them, and scientists are even employing the cells to stir up immunity against cancer.

Cover image by Kenn Brown, Mondolithic Studios.

Articles in this special edition are updated from previous issues of Scientific American.
NEW WEAPONS AGAINST A POTENT ENEMY

64  Taming Vessels to Treat Cancer
by Rakesh K. Jain
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.

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A technique called virotherapy harnesses viruses, those banes of humankind, to stop another scourge—cancer.

80  New Light on Medicine
by Nick Lane
Pigments that turn caustic on exposure to light can fight cancer, blindness and heart disease. Their light-induced toxicity may also help explain the origin of vampire tales.

88  Gaining Ground on Breast Cancer
by Francisco J. Esteva and Gabriel N. Hortobagyi
The newest targeted therapies are helping doctors to tailor increasingly effective treatments to individual patients.
It was February 2003, and Kris Carr, a photographer and actress, was on a roll. The bubbly, green-eyed stunner was in high demand. She was considered “the Julia Roberts of advertising” (at least according to her agent), thanks to her success in two popular Bud Light commercials that aired during the Super Bowl. She also had some impressive theater and film credits, among them a role in Arthur Miller’s Mr. Peter’s Connections, in which she performed (in the buff, no less) alongside actor Peter Falk.

Like many of her hip young peers, Carr, then 31, routinely burned the candle at both ends. She existed on energy bars, fast food and coffee downed between nonstop auditions and takes. Every so often her frenetic lifestyle would catch up with her as it did now: she had just returned home to New York City after “partying like a rock star” at Florida’s Sarasota Film Festival, where a film she had appeared in premiered, and she was dragging. Time to detox, cleanse her body and soul, exercise and eat right for a spell. She swore off drinking for a month and took a vigorous Jivamukti-style yoga class to kick-start her new get-healthy-quick scheme.

“The following morning I woke up feeling like I was hit by a truck,” Carr

By Lisa Stein

Overview

- Rather than surrendering to despair and impersonal medical treatments, growing numbers of cancer patients are empowering themselves with information and control over their therapies. The trend is finding acceptance in mainstream medicine and helping people with cancer lead healthier lives.
- The experiences of author and filmmaker Kris Carr, who was diagnosed with a rare, incurable malignancy, illustrate how successfully one can manage cancer as a chronic disease.
- The following resource guides offer tips on developing a strategy for managing the illness, asking the right questions of physicians and getting the right professional and personal support.
“I DON’T THINK anyone has a better life than me,” says cancer patient Kris Carr.

says. Every muscle ached. She dismissed her sore body as a sign that she was more out of shape than she had thought and, as usual, slipped into tight jeans, slathered on a mask of makeup and headed to an audition: a commercial for a diet shake. (She didn’t get it: too fat, says the slender onetime model.)

By evening, stiff muscles were the least of Carr’s problems. Her pain had worsened, and it was now accompanied by shortness of breath and severe abdominal cramping. She made an appointment to see her doctor the following day.

Gallbladder trouble, the physician surmised after a quick examination. Recommended treatment: yank the pear-shaped organ that, when healthy,
helps the liver flush fats from the body but, when faulty, causes excruciating pain. He gave Carr a prescription for painkillers and sent her for an ultrasound to confirm that her gallbladder was indeed the culprit.

It wasn’t. “When they did the ultrasound, they found the ‘lesions.’ They could see there were spots all over my liver—so many that it looked like Swiss cheese,” Carr says. She was concerned but still blissfully ignorant of the potential ramifications. “I didn’t know,” she says, “that lesions meant tumors.”

A battery of tests over the next few days revealed that Carr was suffering from epithelioid hemangioendothelioma (EHE), a vascular cancer in the lining of the blood vessels in her liver and lungs so rare that only 0.01 percent of the cancer population has it. Around 200 to 300 cases are diagnosed nationwide every year. The cause: unknown. The cancer was stage IV—incurable and inoperable, the doctor said. “Some people say it could have come on like a meteor shower,” Carr says; others suspect the tumors had been developing her whole life.

EHE is typically a slow-moving cancer. There are studies under way but currently no cures or definitive treatments. The doctor recommended a “watch and wait” approach. That is, they take their cues from the tumors—monitor them for two months to gauge whether they were holding steady or moving slowly or swiftly. They were quiet for now, “indolent” in cancer-speak, and the hope was they would stay that way.

It was February 14. “Happy Valentine’s Day. You have cancer,” Carr wrote in her journal that night.

Questions to Ask

Studies show that cancer (and other) patients who arm themselves with information typically fare better and experience fewer side effects than those who simply follow doctors’ orders, no questions asked. Being informed gives them some control over their disease—and that feeling of empowerment plays a role in the healing process. No. 1 rule: do not be cowed by your doctor. Ask him or her to explain anything and everything you don’t understand. Prepare questions in advance of appointments (to reduce stress and the odds of forgetting any) and bring a notebook to jot down answers and other important info. Below are some questions you should ask:

- What causes this type of cancer?
- What are the risk factors? If it’s genetic, are other family members at risk?
- What lifestyle changes (diet, exercise, rest) do you recommend?
- What are my treatment options?
- Are there activities that should be avoided because they might trigger or exacerbate symptoms?
- What happens if new symptoms crop up or existing ones worsen?
- What medical tests or procedures are necessary? How often?
- What stage is my cancer? What does that mean?
- What is my overall prognosis or chance of recovery?
- What are the average survival and cure rates?
- Could my disease go into remission?
- What is the recommended treatment?
- How often will I have to undergo treatment—and for how long?
- What are the potential side effects?
- What are the benefits versus the risks of each treatment option?
- Are there alternative therapies? What are they?
- What are the expected results of treatment?
- Is the treatment painful? If so, is there a way to make it more bearable?
- How long is the recovery? Will it require a hospital stay?
- When can I resume my normal activity (if it’s been curtailed)?
- Has my cancer spread? If so, how does this change treatment decisions?
- Am I eligible for any clinical trials?
- What happens if my disease progresses while I’m in a clinical trial?
- Who foots the bills if I participate in a clinical trial?
- Where can I find emotional, psychological and spiritual support?
- Whom should I call with questions or concerns after office hours?
- May I contact you or a nurse if I have questions or more symptoms? (If the answer is “no,” find another doctor.)
inside her? “How the hell could I do that? How could I live with cancer without thinking of dying every day?” she wondered.

Well, he offered, she could try to strengthen her immune system through diet and lifestyle changes.

“He did not know it, but in that moment he planted the seeds for personal revolution,” Carr says. “I was not going to kick back and wait for the unknown. I was going to dive in and become a full-time healing junkie.”

She set about trying to find out everything she possibly could about cancer. She sought second, third and fourth opinions. “If I had listened to one of the first doctors I talked to, I would have ended up sliced, fried and hauling around not one but three organs that didn’t belong to me,” she says.

Becoming a “Healing Junkie”

Carr hit the books and the Internet. (“I tell people I have a Ph.D. from Google University,” she says, laughing.) She traded in fast food for a vegan diet and swapped martinis for a green brew of cucumbers, kale, celery and sprouts. She formed a “posse” with other young women with cancer. She explored alternative therapies, including massage and meditation, and even spent time in a Zen monastery. And she began the empowering process of documenting and filming her journey—ev-

with cancer without thinking of dying every day?”

Becoming a “Healing Junkie”

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Your Odds of Beating Cancer

Success in the battle against cancer is often measured in terms of the “five-year relative survival rate.” That rate is the number of patients who are still alive five years after being diagnosed, relative to the number who would be expected to survive if they had not come down with the disease. Five years might not seem like a lot, but it is, considering that 67 is the median age for diagnosis.

Below is a sampling of five-year relative survival rates for common types of cancer diagnosed between 1996 and 2004. These rates are calculated by the National Cancer Institute’s Surveillance, Epidemiology and End Results (SEER) program, which collects survival data from state registries covering about 26 percent of the U.S. population.

Survival rates have increased dramatically over the years, thanks to earlier detection and better treatments. The five-year relative survival rate for patients diagnosed with any type of cancer in 1975 was 50 percent; the rate jumped to 67 percent in 2000.

Bear in mind that survival rates vary widely depending on the type of cancer and the patient’s age, gender, general health, lifestyle and ethnicity. You can find more detailed statistics at http://seer.cancer.gov

Five-Year Survival Rates (percent)

- Prostate: 99
- Melanoma (skin): 91
- Breast: 89
- Endometrium: 83
- Urinary bladder: 80
- Kidney: 67
- Non-Hodgkin’s lymphoma: 65
- Colon and rectum: 64
- Ovary: 46
- Lung and bronchus: 15
- Pancreas: 5

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Getting Support: Tips, Tools and Tenderness

You’ve just been diagnosed with cancer. Now what? First and foremost, do not try to handle this on your own. Allow family and friends to help, and find others in your situation to lean on.

Online resources:
- www.cancercare.org: Need a professional cancer assistant? Try the next best thing. This site is designed to help patients navigate their way through cancer—answering questions, finding help or just “listening” when they need to vent.
- http://hippocrateshealthinstitute.com: Site of the Hippocrates Health Institute, a world-renowned healing center in Florida.
- www.mercola.com: An alternative medicine and education site.
- www.heardsupport.org: This site is specifically geared toward patients with hemangiendothelioma, the rare cancer that Carr has.
- www.livestrong.org: Site of seven-time Tour de France winner and cancer survivor Lance Armstrong.
- www.ulmanfund.org: Provides support programs and resources for patients and their families. Also helpful: a downloadable book penned by founders Doug and Diana Ulman.
- www.cancer.gov: This site of the National Cancer Institute is a comprehensive source of state-of-the-art treatments and clinical trials (including a database of open trials).
- www.imtooyoungforthis.org: An invaluable source of support and research for survivors in their 20s and 30s and their families.
- www.cancersurvivorunite.org: Camps and support programs for young adults with cancer.
- www.youngcancerspouses.org: A site designed to connect couples dealing with the ups and downs of cancer.
- www.americancancersociety.com: This American Cancer Society site provides basic information, alternative therapies, ways to manage the disease, and support programs.
- www.oncolink.com: This University of Pennsylvania site offers key cancer info and pointers.
- www.cancerguide.org: A how-to on researching your disease, searching for clinical trials, and finding out about the latest traditional and alternative therapies.
- www.cancer.net: American Society of Clinical Oncology site provides oncologist-approved information to help patients make informed decisions about their health care.
- www.gildasclub.org: Named for Saturday Night Live comedian Gilda Radner, who died of ovarian cancer, this site provides a support network for patients and their families.
- www.thewellnesscommunity.org: The Wellness Community provides support and education for cancer patients and caretakers—and hooks them up with others going through the same thing. It provides info on local wellness communities and even offers a virtual wellness community in Spanish.

“...every thing and everyone she met, from the physicians to the gurus to the quacks. (Beware of quick fixes, she warns: “If anyone offers guarantees—run!”)

She conducted her search for an oncologist as though she were CEO of a company that she dubbed Save My Ass Technologies, Inc., treating prospective doctors as though they were job applicants. “If it was the perfect fit: fine,” she says. “If not: next!” She nixed some of the candidates for their poor bedside manner (“There should be mutual respect”), others because of their proposed treatment plans. Among the dismissed: the one who recommended a triple organ transplant (her liver and both lungs). “Some doctors are still caught up in the old model of muke it and cut it out—and sometimes it is really not necessary.... In my case it was not the protocol,” Carr says. “Do you want them to be stabbing at you if they’re taking that stab in the dark? It’s important to make sure you’re in the right hands. They can help you, or they can kill you. It’s that simple.”

The more physicians she interviewed, the more she came to realize that “half the time they don’t have the answers,” but it is the ones willing to admit that fact who hold the most promise of finding them. Enter the doctor she “hired”: George Demetri, director of the Center for Sarcoma and Bone Oncology at the Dana-Farber Cancer Institute in Boston, who, unlike many of the other “job applicants,” not only has the medical credentials but, she says, is also “kind and compassionate” and welcomes his patients’ input.

Keeping Tumors at Bay

Carr says Demetri believes that she can live her “whole life” with the disease but that it may have to be treated with drugs at some point. “We don’t know. There is currently no cure,” she notes, “but there’s no doubt in my mind that any new information, drugs, and
Finding a doctor who specializes in cancer care and choosing a treatment facility are essential steps in any patient’s recovery program. One good place to start is with the 63 cancer centers that the National Cancer Institute recognizes for “scientific excellence and the capability to integrate a diversity of research approaches” [http://cancercenters.cancer.gov/cancercenters]. You can also check whether the American College of Surgeons’ Commission on Cancer [www.facs.org/cancerprogram] approves of a given program. Some of the things to look for in a cancer center include a low mortality index, a high ratio of nurses to patients and opportunities to participate in clinical trials. For more tips, see www.cancer.gov/cancertopics/factsheet/Therapy/doctor-facility. Here is a selection of some of the most respected cancer treatment centers around the country:

**Medical Resources**

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<tr>
<th>Center</th>
<th>City</th>
<th>Phone</th>
<th>Website</th>
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<tbody>
<tr>
<td>Dana-Farber Cancer Institute</td>
<td>Boston</td>
<td>866-408-DFCI</td>
<td><a href="http://www.dfci.harvard.edu">www.dfci.harvard.edu</a></td>
</tr>
<tr>
<td>Memorial Sloan-Kettering Cancer Center</td>
<td>New York City</td>
<td>212-639-2000</td>
<td><a href="http://www.mskcc.org">www.mskcc.org</a></td>
</tr>
<tr>
<td>Duke Comprehensive Cancer Center</td>
<td>Durham, N.C.</td>
<td>888-ASK-DUKE</td>
<td><a href="http://www.cancer.duke.edu">www.cancer.duke.edu</a></td>
</tr>
<tr>
<td>University of Texas M. D. Anderson Cancer Center</td>
<td>Houston</td>
<td>877-MDA-6789</td>
<td><a href="http://www.mdanderson.org">www.mdanderson.org</a></td>
</tr>
<tr>
<td>University of Chicago Medical Center</td>
<td>Chicago</td>
<td>888-UCH-0200</td>
<td><a href="http://www.uchospitals.edu/specialties/cancer">www.uchospitals.edu/specialties/cancer</a></td>
</tr>
<tr>
<td>University of Washington Medical Center</td>
<td>Seattle</td>
<td>206-598-4100</td>
<td><a href="http://www.uwmedicine.washington.edu/PatientCare/MedicalSpecialties/SpecialtyCare/UWMEDECALCENTERCancer">www.uwmedicine.washington.edu/PatientCare/MedicalSpecialties/SpecialtyCare/UWMEDECALCENTERCancer</a></td>
</tr>
<tr>
<td>UCLA Medical Center</td>
<td>Los Angeles</td>
<td>888-UCLA-MD1</td>
<td><a href="http://www.uclahealth.org">www.uclahealth.org</a></td>
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“Treatment is going to come out of this place [Dana-Farber]. I’m in the right place to be monitored.”

Four years after turning the camera on herself, Carr turned her healing journey into a documentary called Crazy Sexy Cancer, which TLC bought in the fall of 2006. Last year it had its world premiere at the South by Southwest Film Festival in Austin, Tex.

“I’m not saying that cancer is sexy,” she stresses. “What I’m saying is that we are still empowered. We are still alive and whole. I might have cancer, but I’m dealing with it and I’m still all that. The most important thing is to have a voice and use it.”

Carr is among a growing num-
ber of people living and thriving with cancer, thanks to medical advances as well as a progressive philosophy in oncology that recognizes past mistakes of overtreatment and welcomes alternative medicine as a partner in the healing process. The new approach, she says, shatters the stigma that cancer is either a death sentence or something that has to be eradicated—and opens the door to treatments designed to keep tumors in check, which could buy time while new therapies are developed. “Many amazing new treatments are targeting tumors and leaving patients with their lives and their immune systems intact,” she says. “Plus, there is so much that we as patients can do to help our bodies regain health.”

Carr is currently developing a non-profit organization that will work with top oncologists on studies and research using data from the more than 1,000 members of her online community (www.crazysexylife.com) and the 5,000 to 10,000 people who visit her Web site (www.crazysexycancer.com) every week. “We want to be the bridge, one of many bridges, between Western and alternative medicine,” she says.

When first diagnosed, Carr viewed cancer as a freight train to death; now she views it as a “catalyst” for change. She changed her lifestyle, met a new community of women and ditched acting for writing, something she never believed she could do. Last year she wrote and published *Crazy Sexy Cancer Tips* (Globe Pequot Press), a book chock-full of practical advice on everything from doctor shopping to diet to how to keep your wits about you when diagnosed with the Big “C” (or any other disease, for that matter). She wrote a companion book, *Crazy Sexy Cancer Survivor: More Rebellion and Fire for Your Healing Journey*, due out in September—and is set to pen a diet and lifestyle manual to be published next year.

Perhaps most important, she says, cancer led her to her “soul mate.” She recruited Brian Fassett to help her film, edit and produce her documentary. During the project, they fell in love—and Fassett and Carr (who, when first diagnosed, thought she would never date...)

“Once I was able to change my focus, I was able to work as a team with my doctors, and that’s really changed my whole life.”

How to Stay Healthy

Patients undergoing treatment can shore up their physical (and emotional) reserves by eating well, exercising and cutting stress (which impairs the immune system). The American Institute for Cancer Research, which funds studies on the role of food and exercise in cancer prevention and treatment, recommends a diet that’s at least two-thirds vegetables, fruit, whole grains and beans. Below is a roundup of research related to staying healthy:

- A study of 22,000 healthy Greeks showed their “Mediterranean diet,” rich in vegetables, whole grains, olive oil, fruit and fish, reduced their risk of dying from cancer by at least 25 percent. Other studies have found that nutrients in dark, leafy greens may inhibit the growth of tumor cells in breast, skin, lung and stomach cancers and that green tea may thwart cancer development in colon, liver, breast and prostate cells. (A leading theory: flavonoids in tea and carotenoids in leafy greens, which act as antioxidants, may protect against cancer by rooting out free radicals.)

- A pair of 2006 studies showed that regular exercise reduced by up to 61 percent the odds of death in colorectal cancer patients. The findings held even in patients who did not start exercising until after diagnosis.

- A 2005 study showed that 92 percent of nearly 3,000 women with breast cancer who walked or did other exercise three to five hours weekly were still alive 10 years after their diagnosis, compared with 86 percent of those who exercised less than an hour a week.

- A 30-year review of the scientific literature, published in 2004, suggested that cancer patients who feel helpless or who suppress negative emotions may be at greater risk of having their cancer spread than those who play a role in their healing.
desperation led to inspiration.”

Again, let alone marry) got hitched in the fall of 2006. “It was one of the happiest days of my life,” she says. “We vowed to be fellow adventurers. We thought it would be way too melodramatic to say ‘till death do us part.’ This was a day that cancer just was not a part of.” They are now considering having kids. (“Will the hormones wake the sleeping dragon? We don’t know,” she says, “but I refuse to live my life in fear.”) And they have started their own production company, Red House Pictures.

So how is the 36-year-old Carr today, more than five years since her life-altering diagnosis? “I am happy and, I think, healthier than I was before I was diagnosed.” Her last scan in February showed the tumors are stable.

Looking back on her healing journey, she muses: “The doctors told me to ‘watch and wait.’ What I prefer is the ‘watch and live’ approach. I’m not waiting, putting my life on hold. I’m living my life, just with the knowledge that cancer is in my body.

“I think that life is just too sweet to be bitter. Once I was able to change my focus, desperation led to inspiration. I made so many changes, and I thought: This is an awesome life. I mean, honestly, I don’t think anyone has a better life than me. How can you live with the knowledge of cancer? I might not ever be able to get rid of it, but I can’t let that ruin my life… I think: Just go for it. Life is a terminal condition. We’re all going to die. Cancer patients just have more information, but we all, in some ways, wait for permission to live.”


Looking Ahead: Start a Family?

Does a cancer diagnosis spell the end of your dreams to have a family? In a word—no. Note to readers: check your options before undertaking treatments that may cause infertility. In the event that you cannot become pregnant, there is always surrogacy and adoption. Despite what you’ve heard, it is possible to adopt if you’ve had cancer. The key: pick an agency and country that are open to working with cancer survivors.

For more, check out:

- www.fertilehope.org: This site provides unvarnished facts about fertility risks associated with cancer treatment as well as fertility-preservation and parenthood alternatives before, during and after treatment. It outlines the success rates, costs and time requirements for a variety of fertility procedures and also addresses other possibilities, including egg and sperm donation, surrogacy and adoption.

- www.pregnantwithcancer.org: This Web site links newly pregnant cancer patients with others with a similar cancer who have already been there, done that.
EvolvEd for Cancer?

By Carl Zimmer
Natural selection lacks the power to erase cancer from our species and, some scientists argue, may even have provided tools that help tumors grow.

**NATURAL SELECTION IS NOT NATURAL PERFECTION.**

Living creatures have evolved some remarkably complex adaptations, but we are still very vulnerable to disease. Among the most tragic of those ills—and perhaps most enigmatic—is cancer. A cancerous tumor is exquisitely well adapted for survival in its own grotesque way. Its cells continue to divide long after ordinary cells would stop. They destroy surrounding tissues to make room for themselves, and they trick the body into supplying them with energy to grow even larger. But the tumors that afflict us are not foreign parasites that have acquired sophisticated strategies for attacking our bodies. They are made of our own cells, turned against us. Nor is cancer some bizarre rarity: a woman in the U.S. has a 39 percent chance of being diagnosed with some type of cancer in her lifetime. A man has a 45 percent chance.
These facts make cancer a grim yet fascinating puzzle for evolutionary biologists. If natural selection is powerful enough to produce complex adaptations, from the eye to the immune system, why has it been unable to wipe out cancer? The answer, these investigators argue, lies in the evolutionary process itself. Natural selection has favored certain defenses against cancer but cannot eliminate it altogether. Ironically, natural selection may even inadvertently provide some of the tools that cancer cells can use to grow.

The study of cancer evolution is still in its infancy, with much debate about the mechanisms involved and much testing of hypotheses left to carry out. Some medical researchers remain skeptical that the work will affect the way they fight the disease. Evolutionary biologists agree that they are not about to discover a cure for cancer, but they argue that understanding cancer’s history could reveal clues that would otherwise remain hidden. “Obviously, we always have that in the back of our minds in everything we do,” says Judith Campisi of Lawrence Berkeley National Laboratory.

The Dawn of Cancer
AT ITS ROOT, cancer is a disease of multicellularity. Our single-celled ancestors reproduced by dividing in two. After animals emerged, about 700 million years ago, the cells inside their bodies continued to reproduce by dividing, using the molecular machinery they inherited from their progenitors. The cells also began to specialize as they divided, forming different tissues. The complex, multicellular bodies animals have today were made possible by the emergence of new genes that could control how cells divided—such as by stopping the cells’ reproduction once an organ reached its adult size. The millions of animal species are evidence of the great evolutionary success that came with acquiring a body. But bodies also present a profound risk. Whenever a cell inside a body divides, its DNA has a small chance of acquiring a cancer-causing mutation. “Every time a cell divides, it’s going to be at risk of developing into cancer,” Campisi says.

Rare mutations, for instance, may cause a cell to lose restraint and begin to multiply uncontrollably. Other mutations can add to the problem: They may allow deranged cells to invade surrounding tissues and spread through the body. Or they may allow tumor cells to evade the immune system or attract blood vessels that can supply fresh oxygen.

Cancer, in other words, re-creates within our own bodies the evolutionary process that enables animals to adapt to their environment. At the level of organisms, natural selection operates when genetic mutations cause some organisms to have more reproductive success than others; the mutations get “selected” in the sense that they persist and become more common in future generations. In cancer, cells play the role of organisms. Cancer-causing changes to DNA cause some cells to reproduce more effectively than ordinary ones. And even within a single tumor, more adapted cells may outcompete less successful ones. “It’s like Darwinian evolution, except that it happens within one organ,” explains Natalia Komarova of the University of California, Irvine.

Limits to Defenses
ALTHOUGH OUR BODIES may be vulnerable to cancer, they also have many ways to halt it. These strategies probably resulted from natural selection, because mutations that made our ancestors less likely to die of cancer in their prime could have raised their reproductive success. But given the many millions of people who get cancer every year, it is obvious that these defenses have not eradicated the disease. By studying the evolution of these defenses, biologists are trying to understand why they fall short.

Tumor suppressor proteins are among the most effective defenses against cancer. Studies suggest that some of these proteins prevent cancer by monitoring how a cell reproduces. If the cell multiplies in an abnormal way, the proteins induce it to die or to slip into senescence, a kind of early retirement. The cell survives, but it can no longer divide.

Overview/Cancer Evolution

- Natural selection has only a limited ability to prevent cancer. It has provided some defenses, but these tend to delay the disease until late in life rather than eliminating it entirely.
- In addition, evolutionary forces have apparently favored some genes that can contribute to cancer’s development or aggressiveness.
- An understanding of cancer’s evolutionary history—and how individual tumors evolve in the body—could suggest fresh angles of attack on the disorder.
Tumor suppressor proteins play a vital role in our survival, but scientists have recently discovered something strange about them: in some respects, we would be better off without them.

Norman E. Sharpless of the University of North Carolina at Chapel Hill genetically engineered mice to study the effect of one of these proteins, called p16 (or, more properly, p16-Ink4a). He and his colleagues created a line of mice that lacked a functional gene for p16 and thus could not produce the protein. In September 2006 the group published three studies on the mice. As expected, the animals were more prone to cancer, which could arise when they were only a year old.

But losing the p16 gene had an upside. When the mice got old, their cells still behaved as if they were young. In one experiment, the scientists studied older mice, some of which had working p16 genes and some of which did not. They destroyed insulin-producing cells in the pancreases of the animals. The normal rodents could no longer produce insulin and developed fatal diabetes. But the ones without the p16 protein developed only mild diabetes and survived. The progenitors of their insulin-producing cells could still multiply quickly, and they repopulated the pancreas with new cells. The scientists found similar results when they examined cells in the blood and brains of the mice: p16 protected them against cancer but also made them old.

These results support a hypothesis Campisi has developed over the past few years. Natural selection favors anticancer proteins such as p16, but only in moderation. If these proteins become too aggressive, they can create their own threats to health by making bodies age too quickly. “It’s still a working hypothesis,” Campisi admits, “but the data are looking stronger and stronger.”

Delays the Inevitable

A defense against cancer does not have to eradicate the disease completely to be favored by natural selection. If it can just delay tumors until old age, it may allow people to have more children, on average, than others who lack the defense. It may seem cruel for evolution to stick old people with cancer, but as Jarle Breivik of the University of Oslo points out, “natural selection does not favor genes because they let us live long and happy lives. They are selected for their ability to propagate their information through the generations.”

Anticancer proteins such as p16 may favor the young over the old. When p16 pushes a cell into senescence, the cell does not just stop multiplying. It also begins producing an odd balance of proteins. Among the proteins it makes is vascular endothelial growth factor (VEGF), which triggers the growth of more blood vessels. VEGF fosters the growth of tumors by supplying them with extra nutrients. In young people, p16’s main effect may be to suppress cancerous cells. But over time, it may create a growing population of senescent cells, which could make people more vulnerable to cancer in old age.

Another way to delay cancer is to set up several lines of defense. Studies on colon cancer, for example, show that cells in the colon must acquire mutations to several genes before they turn cancerous. These defense lines do not prevent people from getting colon cancer—in fact, it is the third most common form of the disease. But the need for multiple mutations to occur in a cell may reduce the chances that colon cancer will arise in young individuals. The average age of people diagnosed with colon cancer is 70.

Not all cancers strike the old, of course. Most victims of a cancer of the retina called retinoblastoma, for example, are children. But Leonard Nunney of the University of...
California, Riverside, argues that evolution is responsible for that difference between the two cancers. He points out that colon cells have many more opportunities for acquiring dangerous mutations than retinal cells do. The colon is a large organ made of many cells, which continue replicating throughout a person’s life as old cells slough off and new ones take their place. That risk puts a big evolutionary premium on defenses that can prevent colon cells from turning cancerous.

The retina, on the other hand, is “the smallest bit of tissue you can imagine,” as Nunney puts it. That small set of retinal cells also stops multiplying by the time a child turns five. With fewer cell divisions occurring, the retina has far fewer opportunities to turn cancerous. As a result, retinoblastoma is extremely rare, striking only four people in a million. Because the risk is so much lower, Nunney argues, natural selection cannot drive the spread of new defenses against retinoblastoma. A defense against cancer in the retina would make very little difference to the average reproductive success of a population.

Scientists suspect that the adaptive advantages brought by these genes outweigh the harm they may cause. One of these highly evolved cancer genes makes a protein called fatty acid synthase (FAS). Normal cells use the protein encoded by this gene to make some of their fatty acids, which are used for many functions, such as building membranes and storing energy. In tumors, however, cancer cells produce FAS protein at a much higher rate. The protein is so important to them that blocking the activity of the gene can kill cancer cells.

By comparing the sequence of the FAS gene in humans and other mammals, Mary J. O’Connell of Dublin City University and James McInerney of the National University of Ireland found that the gene has undergone strong natural selection in humans. “This gene has really changed in our lineage,” McInerney says. McInerney cannot say what FAS does differently in humans, but he is intrigued by a hypothesis put forward by the late psychiatrist David Horrobin in the 1990s. Horrobin argued that the dramatic increase in the size and power of the human brain was made possible by the advent of new kinds of fatty acids. Neurons need fatty acids to build membranes and make connections. “One of the things that might allow a larger brain size was our ability to synthesize fats,” McInerney speculates. But with that new ability may have come a new tool that cancer cells could borrow for their own ends. Cancer cells may, for example, use FAS as an extra source of energy.

Many fast-evolving cancer genes normally produce proteins in tissues involved in re-
production—in the placenta, for instance. Bernard J. Crespi of Simon Fraser University in British Columbia and Kyle Summers of East Carolina University argue that these genes are part of an evolutionary struggle between children and their mothers.

Natural selection favors genes that allow children to draw as much nourishment from their mothers as possible. A fetus produces the placenta, which grows aggressively into the mother’s tissue and extracts nutrients. That demand puts the fetus in conflict with its mother. Natural selection also favors genes that allow mothers to give birth to healthy children. If a mother sacrifices too much in the pregnancy of one child, she may be less likely to have healthy children afterward. So mothers produce compounds that slow down the flow of nutrients into the fetus.

Each time mothers evolve new strategies to restrain their fetuses, natural selection favors mutations that allow the fetuses to overcome those strategies. “It’s a restrained conflict. There’s a tug-of-war about how much the fetus is going to take from the mother,” Crespi says.

Genes that allow cells to build a better placenta, Crespi and Summers argue, can get hijacked by cancer cells—turned on when they would normally be silent. The ability to stimulate new blood vessel formation and aggressive growth serves a tumor just as it does a placenta. “It’s something naturally liable to be co-opted by cancer cell lineages,” Summers says. “It sets up the opportunity for mutations to create tools for cancer cells to use to take over the body.”

Yet even though activation of these usually quiet genes may make cancers more potent, natural selection may still have favored them because they helped fetuses grow. “You may get selection for a gene variant that helps the fetus get a little more from mom,” Crespi says. “But then, when that kid is 60, it might increase the odds of cancer by a few percent. It’s still going to be selected for because of the strong positive early effects.”

Sperm are another kind of cell that multiplies rapidly. But whereas placental cells proliferate for a few months, sperm-making cells function for a lifetime. “For decades, human males are producing an enormous amount of sperm all the time,” says Andrew Simpson of the Ludwig Institute for Cancer Research in New York City. Genes that operate specifically in such cells are also among the fastest evolving in the human genome. A gene that allows a progenitor sperm cell to divide faster than other cells will become more common in a man’s population of sperm. That means it will be more likely to get into a fertilized egg and be passed down to future generations.

Unfortunately for us, genes that make for fast-breeding sperm cells can make for fast-

Evolution of a Cancer-Causing Virus

The American Cancer Society estimates that 17 percent of all cancer cases—more than 1.8 billion a year—are caused by viruses and other infectious agents. Scientists are studying the evolution of these cancer-causing pathogens to find hints for fighting them. One such pathogen is the human papillomavirus, responsible for most of the half a million cases of cervical cancer diagnosed annually. The virus can cause host cells to divide long after normal cells would stop and also prevents them from repairing mutations to their DNA.

Scientists have reconstructed some of the virus’s evolutionary history by sequencing and comparing the genomes of hundreds of different types of viruses. Papillomaviruses, which form a large family, are found in most vertebrates, in whom they typically engender only warts and other benign growths. Yet when *Homo sapiens* first emerged—about 200,000 years ago in Africa—our ancestors already carried a number of strains that could infect our species and no other animal, and these included cancer-causing types.

After about 100,000 years, *H. sapiens* expanded out of Africa to other continents, bringing the viruses with them. As human populations became isolated from one another, their papillomaviruses did as well. Consequently, the genealogy of human papillomaviruses reflects human genealogy. The oldest lineage of the viruses is most common in living Africans, for example. Native Americans descended from Asians, and their viruses share that kinship.

This coevolution may be medically relevant, because the viruses appear to have adapted to their hosts. In August 2006 scientists published a report in the *Journal of the National Cancer Institute* on the persistence of various virus types in different ethnic groups. A woman who becomes infected by a virus having an ancient association with her ethnic group will carry the virus for a longer time than if she were infected by another type.

Scientists are also investigating how certain benign papillomaviruses evolved to cause cancer. Their discoveries will become all the more important as vaccines are introduced against the viruses. The FDA has approved a vaccine against the most dangerous human papillomavirus strain, known as *H*.16. But evolutionary studies indicate that on rare occasions, human papillomavirus types have traded genes involved in triggering cancer. The global HIV epidemic might raise the risk of this gene swapping. As HIV weakens a person’s immune system, more types of human papillomaviruses can invade and coexist. This mingling could conceivably give rise to a new cancer-causing strain for which today’s vaccines would be less protective.

—C.Z.
breeding cancer cells. Normally, non-sperm cells prevent these genes from making proteins. “These are genes that need to be firmly silenced, because they are dangerous genes,” Simpson says. It appears that in cancer cells, mutations can unlock these sperm genes, allowing the cells to multiply quickly.

How vs. Why

Evolutionary biologists hope that their research can help in the fight against cancer. In addition to clarifying why evolution has not eradicated cancer, evolutionary biology may elucidate one of the most daunting challenges faced by oncologists: the emergence of drug-resistant tumors.

Chemotherapy drugs often lose their effectiveness against cancer cells. The process has many parallels to the evolution of resistance to antiviral drugs in HIV. Mutations that allow cancer cells to survive exposure to chemotherapy drugs enable the tumor cells to outcompete more vulnerable cells. Understanding the evolution of HIV and other pathogens has helped scientists to come up with new strategies for avoiding resistance. Now scientists are investigating how understanding the evolution within tumors could lead to better ways of using chemotherapy.

The concepts evolutionary biologists have been exploring are relatively new for most cancer biologists. Some are reacting with great enthusiasm. Simpson believes, for instance, that deciphering the rapid evolution of sperm-related genes could help in the fight against tumors that borrow them. “I think it’s absolutely crucial to understand exactly why there is such strong selection on these genes,” Simpson says. “Understanding that will give us a real insight into cancer.”

Bert Vogelstein of the Howard Hughes Medical Institute also finds it useful to view cancer through an evolutionary lens. “Thinking about cancer in evolutionary terms jibes perfectly with the views of cancer molecular geneticists,” he says. “In one sense, cancer is a side effect of evolution.”

But Vogelstein is not yet persuaded by the significance of fast-evolving cancer genes: “One has to be a little cautious. The first question I would ask is, Are they looking at the whole genome in a wholly unbiased way?” McInerney acknowledges that such systematic studies have not yet been conducted, but the early results have prompted him and other scientists to begin them.

Some cancer specialists are leery of the entire approach. Christopher Benz of the Buck Institute for Age Research in Novato, Calif., says that no insights from evolution should be accepted until they are put to an experimental test the way any other hypothesis would be. “Call me skeptical,” he says.

Crespi is familiar with this skepticism, and he thinks that it may emerge from the different kinds of questions evolutionary biologists and cancer biologists ask. “The people working on cancer are working on the
Perhaps by asking different questions, evolutionary biologists will be able to contribute to some of the debates among cancer biologists. One long-standing argument focuses on whether mice are good models for cancer in humans. Some evolutionary biologists argue that they are not, because of their separate history. Rodents inherited the same set of genes as we did from our common ancestor some 100 million years ago, but then many of those genes underwent more change in the two lineages. Cancer-related genes such as FAS may have experienced intense evolutionary change in humans in just the past few million years, making them significantly different from their counterparts in mice.

Mice may also be a poor choice for a cancer model because of the way they reproduce. Scientists have bred lab mice to produce more pups at a faster rate than their wild cousins. Such manipulation may have altered the evolutionary trade-off faced by mice, so that they are rewarded for investing energy into growing quickly and reproducing young. At the same time, this artificial selection may be selecting against cancer defenses. “We have changed their life histories by selecting on their timing of reproduction,” Crespi says.

Ultimately, the study of the evolution of cancer may reveal why eradicating the disease has proved so difficult. “There is no real solution to the problem,” Breivik says. “Cancer is a fundamental consequence of the way we are made. We are temporary colonies made by our genes to propagate them to the next generation. The ultimate solution to cancer is that we would have to start reproducing ourselves in a different way.”

**MORE TO EXPLORE**

If we wish to learn more about cancer, we must now concentrate on the cellular genome.” Nobel laureate Renato Dulbecco penned those words more than 20 years ago in one of the earliest public calls for what would become the Human Genome Project. “We are at a turning point,” Dulbecco, a pioneering cancer researcher, declared in 1986 in the journal Science. Discoveries in preceding years had made clear that much of the deranged behavior of cancer cells stemmed from damage to their genes and alterations in their functioning. “We have two options,” he wrote. “Either try to discover the genes important in malignancy by a piece-meal approach, or … sequence the whole genome.”

Dulbecco and others in the scientific community grasped that sequencing the human genome, though a monumental achievement itself, would mark just the first step of the quest to fully understand the biology of cancer. With the complete sequence of nucleotide bases in normal human DNA in hand, scientists would then need to classify the wide array of human genes according to their function—which in turn could reveal their roles in cancer. Over the span of two decades Dulbecco’s vision has moved from pipe dream to reality. Less than three years after the Human Genome Project’s completion, the National Institutes of Health officially launched the pilot stage of an effort to create a comprehensive catalogue of the genomic changes involved in cancer: The Cancer Genome Atlas (TCGA).

The main reason to pursue this next ambitious venture in large-scale biology with great urgency is cancer’s terrible toll on humankind. Every day more than 1,500 Americans die from cancer—about one person every minute. As the U.S. population ages, this rate is expected to rise significantly in the years ahead unless investigators find ways to accelerate the identification of new vulnerabilities within cancerous cells and develop novel strategies for attacking those targets.

Still, however noble the intent, it takes more than a desire to ease human suffering to justify a research enterprise of this magnitude. When applied to the 50 most common types of cancer, this effort could ultimately prove to be the equivalent of more than 10,000 Human Genome Projects in terms of the sheer volume of DNA to be sequenced. The dream must therefore be matched with an ambitious but realistic assessment of the emerging scientific opportunities for waging a smarter war against cancer.

**A Disease of Genes**

The idea that alterations to the cellular genome lie at the heart of all forms of cancer is not new. Since the first identification in 1981 of a cancer-promoting version of a human gene, known as an oncogene, scientists have increasingly come to understand that cancer is caused primarily by mutations in specific genes. The damage can be incurred through exposure to toxins or radiation, by faulty DNA repair processes or by errors that occur when DNA is copied prior to cell division. In relatively rare cases, a cancer-predisposing mutation is carried within a gene variant inherited from one’s ancestors.

Whatever their origin, these mutations disrupt biological pathways in ways that result in the uncontrolled cell replication, or growth, that is characteristic of cancer as well as other hallmarks of malignancy, such as the abil-
Gene malfunctions underlie the ability of cancer cells to escape normal constraints on a cell’s behavior. Because genes give rise to proteins that serve as cellular building blocks, signals and regulators of other genes, a mutation that disables one gene, or causes it to be overactive, can have multiple deranging effects on the cell (below). Nevertheless, cells usually need to accrete several cancer-promoting, or oncogenic, mutations in separate genes to acquire the hallmark properties of malignancy [box at right]. Identifying all the genes whose alteration can produce those traits should one day reveal which mutations are the true drivers of specific types of cancer—even of a specific patient’s malignancy—and therefore the most effective ways to intervene in the disease.

**Overview/Cancer Connections**

- Changes in the structure or activity of genes underlie the malignant behavior of cancer cells.
- Identification of genes involved in certain cancers is already advancing diagnosis and treatment.
- The Cancer Genome Atlas is a monumental initiative to eventually identify all the genetic alterations in different forms of cancer so that gene changes driving the disease can be targeted directly.

**Complex Circuitry**

The extraordinarily complex molecular interactions in a human cell can be viewed as a network of parallel and intersecting pathways. A simplified depiction (right) of just one such pathway that promotes cell proliferation begins with a family of epidermal growth factor receptors (EGFR) in the cell membrane. Their stimulation by growth factors outside the cell transmits signals to additional proteins and genes, ultimately prompting the cell to “grow” by dividing.

**Oncogenic Mutations**

In a significant portion of lung and breast tumors, members of the EGFR gene family are mutated or duplicated, which boosts the number or function of the receptors they encode, overstimulating this growth pathway. Damage to downstream genes can have similar results. Changes in the B-RAF gene, seen in some 70 percent of melanomas, also promote hyperactive cell proliferation. Versions of the RAS gene are mutated in many cancer types, which can affect cell growth as well as intersecting pathways—for example, interfering with a suicide program that normally destroys damaged cells.
Hallmarks of Cancer

The six abnormal capabilities listed below together give tumors their lethal power to overrun their native tissue and spread through the body.

**Self-sufficiency in growth signaling**
Cancer cells amplify external growth cues or generate their own.

**Invasion and motility**
Cancer cells defy multiple signals and forces that hold a cell in place and prevent it from traveling to—and thriving in—other tissues.

**Evasion of cell suicide**
Mechanisms that should trigger or carry out a self-destruct program in damaged cells are disabled or overridden.

**Limitless replicative potential**
Cancer cells evade intrinsic limits on the number of times a normal cell can divide.

**Sustained blood vessel growth**
Tumors emit signals promoting the development of new blood vessels to deliver oxygen and nutrients.

**Insensitivity to antigrowth signals**
Cancer cells defy multiple signals and forces that hold a cell in place and prevent it from traveling to—and thriving in—other tissues.

**Evasion of cell suicide**
Mechanisms that should trigger or carry out a self-destruct program in damaged cells are disabled or overridden.

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Cancer cells evade intrinsic limits on the number of times a normal cell can divide.

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**Invasiveness and motility**
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Proofs of Concept

Advances in the technologies used to sequence and analyze genomes. At the start of that project in 1990, for example, the cost of DNA sequencing was more than $10 per “finished” nucleotide base. Today the cost is less than a penny per base and is expected to drop further with the emergence of innovative sequencing methods. The late-scale approach embodied in TCGA—unthinkable even a few years ago—has emerged as perhaps the most efficient and cost-effective way to identify the wide array of genomic factors involved in cancer.

Francis S. Collins and Anna D. Barker are leaders of The Cancer Genome Atlas initiative in their positions as, respectively, director of the National Human Genome Research Institute and deputy director for Advanced Technologies and Strategic Partnerships of the National Cancer Institute. Collins led the Human Genome Project to its completion of the human DNA sequence, and Barker has headed drug development and biotechnology research efforts in the public and private sectors, with a particular focus on fighting cancer.
Genes and Cancer

A connection between genetic abnormalities and the aberrant features of cancer cells was first suggested more than 100 years ago by German biologist Theodor Boveri and others. But over the past few decades evidence that gene alterations directly cause the deranged behavior of cancer cells began accumulating. Calls arose by 1986 to sequence the normal human genome to study malignant gene changes comprehensively. The Human Genome Project was completed in 2003. The Cancer Genome Atlas project recently started cataloguing the gene mutations found in three types of cancer.

- **1986**
  Fused gene *BCR-ABL* in the Philadelphia chromosome is found to cause CML.

- **1986**
  First tumor suppressor gene, *RB1*, is identified.

- **1987**
  Altered methylation of DNA, suspected to affect gene activation, is found in cancer cells.

- **1986**
  U.S. Department of Energy considers sequencing the human genome to further study of radiation effects.

- **1976**
  Scientists discover that *src*, a nonviral gene found in animal cells, can cause cancer.

- **1979**
  *P53*, later found to be the most frequently mutated gene in human cancer, is discovered.

- **1981**
  *H-RAS* is the first human oncogene (a gene whose alteration is cancer-promoting) to be discovered.

- **1960**
  First genetic defect associated with a specific cancer—an abnormality known as the Philadelphia chromosome—is discovered in chronic myelogenous leukemia (CML) cells.

- **1950s–1960s**
  Multiple discoveries reveal that tumor viruses can cause cancer by injecting their genes into cells.

- **1990**
  Human Genome Project begins.

- **1990**
  Model of multistep tumor genesis clarifies the role of accumulated gene changes in cellular transformation to malignancy.

- **1990**
  The Cancer Genome Atlas project recently started cataloguing the gene mutations found in three types of cancer.

- **1890–1914**
  Studies of abnormal chromosome distribution during cell division suggest a role in malignancy.

- **1950s–1960s**
  Multiple discoveries reveal that tumor viruses can cause cancer by injecting their genes into cells.

- **1986**
  Renato Dulbecco, writing in *Science*, calls for sequencing the human genome to advance cancer research.

- **1890–1914**
  Theodor Boveri

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Compiling a Colossal Atlas

A phased-in strategy that proved successful at the beginning of the Human Genome Project was to test protocols and technology before scaling up to full DNA sequence production. Similarly, TCGA is beginning with a pilot project to develop and test the scientific framework needed to ultimately map all the genomic abnormalities involved in cancer.

In 2006 the National Cancer Institute and National Human Genome Research Institute selected the scientific teams and facilities that are participating in this pilot project, along with the cancer types they have since begun examining. Over the next few years these two institutes are devoting $100 million to compiling an atlas of genomic changes in three tumor types: glioblastomas of the brain, lung cancer and ovarian cancer. These particular cancers were chosen for several reasons, including their value in gauging the feasibility of scaling up this project to a much larger number of cancer types. Indeed, only if this pilot phase achieves its goals will the NIH move forward with a full-fledged project to develop a complete cancer atlas.

The three malignancies selected for the pilot collectively account for more than 210,000 cancer cases in the U.S. every year and caused an estimated 191,000 deaths in this country in 2006 alone. Moreover, tumor specimen collections meeting the project’s strict scientific, technical and ethical requirements exist for these cancer types. In September 2006 our institutes announced the selection of several biorepositories to provide both tumor and matched samples of normal tissue from the same patients. These repositories, along with other qualifying academic institutions and private hospitals, are delivering materials to a central Biospecimen Core Resource, one of four major structural components in TCGA’s pilot project.

Cancer Genome Characterization Centers, Genome Sequencing Centers and a Data Coordinating Center constitute the project’s other three main elements [see illustration at right], and all these groups collaborate and exchange data openly. Specifically, the seven Cancer Genome Characterization Centers are using a variety of technologies to examine the activity levels of genes within tumor samples and to uncover and catalogue so-called large-scale genomic changes that contribute to the development and progression of cancer. Such alterations include chromosome rearrangements, changes in gene copy numbers and epigenetic changes, which are chemical modifications of the DNA strand that can turn gene activity on or off without actually altering the DNA sequence.

Genes and other chromosomal areas of interest identified by the Cancer Genome Characterization Centers are targets for sequencing by the three Genome Sequencing Centers. In addition, families of genes suspected to be involved in cancer, such as those encoding enzymes involved in cell-cycle control known as tyrosine kinases and phosphatases, are being sequenced to identify genetic mutations or other small-scale changes in their DNA code. At present, we estimate that some 2,000 genes—in each of perhaps 1,500 tumor samples—will be sequenced during this pilot project. The exact numbers will, of course, depend on the samples obtained and what is discovered...
about them by the Cancer Genome Characterization Centers. Both the sequencing and genome characterization groups, many of which were participants in the Human Genome Project, can expect to encounter a far greater level of complexity than that in the DNA of normal cells. Once cells become cancerous, they are prone to an even greater rate of mutation as their self-control and repair mechanisms fail. The genomic makeup of individual cells can therefore vary dramatically within a single tumor, and the integrated teams will need to develop robust methods for efficiently distinguishing the “signal” of a potentially biologically significant mutation from the “noise” of the high background rate of mutations seen in many tumors. Furthermore, tumors almost always harbor some nonmalignant cells, which can dilute the sample. If the tumor DNA to be sequenced is too heterogeneous, some important mutations may be missed.

The extent of these and other challenges should be known soon, as researchers begin to examine the extraordinary sets of multidimensional data being generated by TCGA. Though still in its early phases, the atlas team’s comprehensive analysis of glioblastoma has already identified at least one new gene that may prove to be important in understanding the cause of this deadly form of brain cancer.

Following the lead of the Human Genome Project and other recent medical genomics efforts, scientists are making all these data swiftly and freely available to the worldwide re-

From Genome to Cancer—Why the Time Is Right

By Renato Dulbecco

When in 1986 I suggested a new project directed at identifying all human genes, one of my overriding goals was to find those genes involved in cancer development—a feat I hoped would lead to new tools for cancer research and, ultimately, to new therapies. That original human genome project has now been carried out and has demonstrated its usefulness for the discovery of genes involved in many diseases, including cancer. Moreover, the genome sequencing effort has been extended to other organisms—from bacteria to chimpanzees—and is showing the unity of life by revealing how many genes distant species share.

In the course of this work, new technologies have also provided a much more detailed understanding of the complicated processes by which genes give rise to a variety of functional molecules. An important outcome of this research is the realization that genes do not act alone but are participants in extensive networks of activity within cells. Any change in the functioning of one gene can therefore be accompanied by changes in the workings of multiple genes and proteins involved in the cells’ self-maintenance.

The complexity of this system in normal cells is evident in what we already know about cancer—that it results from the stepwise loss of such cellular self-control, which becomes more and more complete as the disease progresses. That progression is caused only in part by physical alterations, or mutations, in specific genes; mostly it is the result of consequent changes in the activity of many other genes involved in cell regulation. Single genes may therefore be responsible in the initiation of cancer and so potential therapeutic targets. To reach the more advanced stages of these cancers (such as the acute phase of myeloid leukemia or the metastatic phase of other cancers), however, the participation of many other genes is required. Most of them are still unknown.

An exception is the recently observed phenomenon of oncogene addiction in certain tumor cells: despite the presence of numerous mutations to the cellular genome, turning off the activity of one so-called oncogene causes the cells to commit suicide via a mechanism known as apoptosis. But how generally this phenomenon occurs is also unknown. To approach these questions, it will be necessary to have a complete catalogue of the structural and functional alterations of genes and other cellular components that cause the loss of regulation in cancer cells. This process, in turn, will require a complete determination of their connections into networks by computational means—a task for the future.

On the way to this goal, however, many other unanswered questions can be explored by the research community. A possible role for stem cells in cancer, for example, is supported by similarities in the behavior of stem cells and cancer cells: both have an unlimited ability to divide; both are very sensitive to the cellular environment, or niche, in which they grow; and many of the genes known to be active in stem cells are also activated in cancer cells.

The advent of genomics has provided welcome insight into the mechanisms by which normal cells become cancerous, but our picture is still incomplete. The time has come to obtain a truly comprehensive catalogue of the genes involved in cancer, bringing to bear all the power of the new tools of genomics and molecular biology to the problem. The Cancer Genome Atlas project aims to do just that.

Renato Dulbecco is president emeritus of the Salk Institute for Biological Studies and co-recipient of the 1975 Nobel Prize in Physiology or Medicine for discoveries related to the interaction of tumor viruses and the genetic material of the cell.
search community. To further enhance its usefulness to both basic and clinical researchers and, ultimately, health care professionals, TCGA is linking its sequence data and genome analyses with information about observable characteristics of the patient donors and their clinical outcomes. Developing the bioinformatic tools to gather, integrate and analyze those massive amounts of data, while safeguarding the confidentiality of patient information, is therefore another hurdle that must be cleared to turn our vision into reality.

**Uncharted Territory**

**THE ROAD AHEAD** is fraught with scientific, technological and policy challenges—some of which are known and others as yet unknown. Among the uncertainties to be resolved: Will new sequencing technologies deliver on their early promise in time to make this effort economically feasible? How quickly can we improve and expand our toolbox for systematically detecting epigenetic changes and other large-scale genomic alterations involved in cancer, especially those associated with metastasis? How can we harness the power of computational biology to create data portals that prove useful to basic biologists, clinical researchers and, eventually, health care professionals on the front lines? How can we balance intellectual-property rights in a way that promotes both basic research and the development of therapies? The list goes on.

To avoid raising false expectations, we also must be clear about the questions this project will not attempt to answer. Although it will serve as a resource for a broad range of biologic exploration, TCGA is only a foundation for the future of cancer research and certainly not the entire house. And we face the sobering issue of time—something that is in short supply for many cancer patients and their families. As we survey the considerable empty spaces that exist in our current map of genomic knowledge about cancer, the prospect of filling those gaps is both exhilarating and daunting. Scientists and the public need to know up front that this unprecedented foray into molecular cartography is going to take years of hard work and creative problem solving by thousands of researchers from many different disciplines.

Where all this work will lead can only be dimly glimpsed today. In this sense, our position is similar to that of the early 19th-century explorers Meriwether Lewis and William Clark. As they ventured up the Missouri River into the largely uncharted Northwest Territory in 1804, their orders from President Thomas Jefferson were to “take observations of latitude and longitude at all remarkable points…. Your observations are to be taken with great pains and accuracy; to be entered distinctly and intelligibly for others, as well as yourself.”

Although Lewis and Clark did not find the much-longed-for water route across the continent, their detailed maps proved valuable to their fledgling nation in myriad ways that Jefferson could never have imagined. For the sake of all those whose lives have and will be touched by cancer, we can only hope our 21st-century expedition into cancer biology exceeds even Renato Dulbecco’s grandest dreams.

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**Targeting Gene Changes in Cancer**

TCGA pilot project teams will examine the DNA of some 1,500 tumor samples from patients with cancers of the lung, ovaries or brain (glioblastoma), looking for genetic changes. Approximately 2,000 suspect genes in each sample will be sequenced to identify specific mutations. The list of target genes will be tailored to each cancer type and largely determined by what the Cancer Genome Characterization Centers find in the samples, although candidates will also be drawn from categories of genes already associated with cancer.

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**More to Explore**


Untangling the
What causes cancer?

Tobacco smoke, most people would say. Probably too much alcohol, sunshine or grilled meat; infection with cervical papillomaviruses; asbestos. All have strong links to cancer, certainly. But they cannot be root causes. Much of the population is exposed to these carcinogens, yet only a tiny minority suffers dangerous tumors as a consequence.

A cause, by definition, leads invariably to its effect. The immediate cause of cancer must be some combination of insults and accidents that induces normal cells in a healthy human body to turn malignant, growing like weeds and sprouting in unnatural places.

At this level, the cause of cancer is not entirely a mystery. In fact, a decade ago many geneticists were confident that science was homing in on a final answer: cancer is the result of cumulative mutations that alter specific locations in a cell’s DNA and thus change the particular proteins encoded by cancer-related genes at those spots. The mutations affect two kinds of cancer genes. The first are called tumor suppressors. They normally restrain cells’ ability to divide, and mutations permanently disable the genes. The second variety, known as oncogenes, stimulate growth—in other words, cell division. Mutations lock oncogenes into an active state. Some researchers still take it as axiomatic that such growth-promoting changes to a small number of cancer genes are the initial event and root cause of every human cancer.

For the past few years, however, prominent oncologists have increasingly challenged that theory. They argue that it is more useful to think of cancer as the consequence of a chaotic process, a combination of Murphy’s Law and Darwin’s Law: anything that can go wrong will, and in a competitive environment, the most prolific variants will dominate.

Despite that shared underlying principle, the new theories make different predictions about what kind of treatments will work best. Some suggest that many cancers could be prevented altogether by better screening, changes in diet, and new drugs—or even by old drugs, such as aspirin. Other theories cast doubt on that hope.

Marks of Malignancy

A workable theory of cancer has to explain both why it is predominantly a disease of old age and why we do not all die from it. A 70-year-old is roughly 100 times as likely to be diagnosed with a...
malignancy as a 19-year-old. Yet most people make it to old age without getting cancer.

Biologists estimate that more than 10 million billion cells must cooperate to keep a human being healthy over the course of an 80-year life span. If any one of those myriad cells could give rise to a tumor, why is it that fewer than half the population will ever contract a cancer serious enough to catch a doctor’s attention?

One explanation is that a cell must acquire several extraordinary skills to be malignant. “Five or six different regulatory systems must be perturbed in order for a normal cell to grow as a cancer,” asserts Robert A. Weinberg of the Whitehead Institute at the Massachusetts Institute of Technology. In a 2002 review paper, he and William C. Hahn of the Dana-Farber Cancer Institute in Boston argued that all life-threatening cancers manifest at least six such deranged abilities. (Although Weinberg is one of the founding proponents of the standard paradigm, even those who challenge that theory tend to agree with this view.)

For example, cancer cells continue dividing in situations in which normal cells would quietly wait for a special chemical signal—say, from an injured neighbor. Somehow they counterfeit these progrowth messages. Conversely, tumor cells must ignore “stop dividing” commands that are sent out by the adjacent tissues they squeeze and by their own internal aging mechanisms.

All cancerous cells have serious problems of some sort with their DNA, and as they double again and again, many cells in the resulting colony end up far from the blood vessels that supply oxygen and nutrients. Such stresses trigger autodestruct mechanisms in healthy cells. Tumor cells find some way to avoid this kind of suicide. Then they have to persuade nearby blood vessels to build the infrastructure they need to thrive.

A fifth derangement that almost all cancers acquire is immortality. A culture of normal human cells stops dividing after 50 to 70 generations. That is more than enough doublings to sustain a person through even a century of healthy life. But the great majority of cells in tumors quickly die of their genetic defects, so those rare few that survive must reproduce indefinitely if the tumor is to grow. The survivors do so in part by manipulating their telomeres, gene-free complexes of DNA and protein that protect the ends of each chromosome.

Tumors that develop these five faculties are trouble, but they are probably not deadly. It is a sixth property, the ability to invade nearby tissue and then metastasize to distant parts of the body, that gives cancer its lethal character. Local invasions can usually be removed surgically. But nine of every 10 deaths from the disease are the result of metastases.

Only an elite few cells in a tumor seem to acquire this ability to detach from the initial mass, float through the circulation and start a new colony in a different organ from the one that gave birth to them. Unfortunately, by the time they are discovered, many cancers have already metastasized—including, in the U.S., 76 percent of lung cancers, 55 percent of colorectal cancers, and 37 percent of breast cancers. By then the prognosis is frequently grim.

The Order of Disorder

Doctors could catch incipient tumors sooner if scientists could trace the steps that cells take down the road to cancer after the initial assault to their DNA by a carcinogen or some random biochemical mishap. Researchers broadly agree on the traits of the diseased cells that emerge from the journey. It is the propelling force and the order of each milestone that are under active debate.

The dominant paradigm for 30 years has been that tumors grow in spurts of mutation and expansion. Genetic damage to a cell deletes or disrupts a tumor suppressor gene—RB, p53, BRCA2 and APC are among the best known—thereby suppressing proteins that normally ensure the integrity of the genome and...
the process of cell division. Alternatively, a mutation may increase the activity of an oncogene—such as HER2/NEU, c-fos or c-erbB3—whose proteins then stimulate the cell to reproduce.

Changes to cancer genes free the cell of one or more normal restraints, allowing it to outbreed its neighbors. The cell passes abnormalities in its DNA sequence on to its descendants, which become a kind of clone army that grows to the limits of its capacity. Eventually another random mutation to a cancer gene looses another shackle, initiating another burst of growth.

Cells normally have two copies of every chromosome—one from the mother, the other from the father—and thus two copies, or alleles, of every gene. (In males, the single X and Y chromosomes are notable exceptions.) A mutation to just one allele is enough to activate an oncogene permanently. But it takes two hits to knock out both alleles of a tumor suppressor gene. Four to 10 mutations in the right genes can transform any cell. Or so the theory goes.

The mutant-gene paradigm gained almost universal acceptance because it explained very well what scientists saw in their experiments on genetically engineered mice and human cell cultures. But new technologies now allow researchers to survey large fractions of the genomes of cancerous and precancerous cells taken directly from people. Many recent observations seem to contradict the idea that mutations to a few specific genes lie at the root of all cancers.
Unexplained Phenomena

In 2003, for example, a team led by Michael F. Clarke, then at the University of Michigan at Ann Arbor and now at Stanford University, reported that it had identified distinguishing marks for a rare subset of cells within human breast cancers that can form new tumors. As few as 100 cells of this type quickly spawned disease when injected into mice lacking an immune system. Tens of thousands of other cells, harvested from the same nine breast malignancies but lacking the telltale marks, failed to do so. These were the first tumor-initiating cells ever isolated for solid tumors, and later studies confirmed that for many common human cancers, just a small fraction of the cells in a tumor are responsible for its growth and metastasis—and so for the illness and death of the patient. The discovery of such cells, which some began calling “cancer stem cells,” posed a problem for the mutant-gene theory of cancer. If mutations, which are copied from a cell to its progeny, give tumor cells their deranged powers, then shouldn’t all clones in the army be equally powerful?

In fact, most tumors are not masses of identical clones. On the contrary, closer examination has revealed amazing genetic diversity among their cells, some of which are so different from normal human cells (and from one another) that they might fairly be called new species.

Well over 1,000 genes have been found to be frequently mutated in one kind of cancer or another. According to the standard paradigm, the proteins normally produced by these cancer-related genes, which include tumor suppressor genes [red circles] and oncogenes [green circles], are organized into complex biochemical circuits that control the reproduction and survival of cells. Mutations that cause parts of the circuitry to fail [crosses] or become hyperactive [arrows] prompt cells to multiply into tumors. But the sheer number of cancer genes—only a tiny fraction of which are shown below—have frustrated attempts to deduce which ones are necessary and sufficient to cause the disease.
Moreover, some of the most commonly altered cancer genes have oddly inconsistent effects. Vogelstein’s group has also reported that the much studied oncogenes c-fos and c-erbB3 are curiously less active in tumors than they are in nearby normal tissues. The tumor suppressor gene RB was shown to be hyperactive—not disabled—in some colon cancers, and, perversely, it appears to protect those tumors from their autodestruct mechanisms.

The “two hit” hypothesis—that both alleles of a tumor suppressor gene must be deactivated—has also been upended by the discovery of a phenomenon called haploinsufficiency. In some cancers, it turns out, tumor suppressors are not mutated at all. Their output is simply reduced, and that seems to be enough to push cells toward malignancy. This effect has now been seen for more than a dozen tumor suppressor genes, and investigators expect to find many more like them. Searching for the mere presence or absence of a gene’s protein is too simplistic. Dosage matters.

**Beyond Mutation**

Researchers are now looking more closely at other phenomena, besides errors in a gene’s DNA sequence, that can dramatically alter the dosage of a protein in a cell. The loss or gain of a chromosome (or part of one) containing the gene can do this. So can tweaks to regulatory factors that control how often a gene is translated into protein, as well as epigenetic phenomena that alter gene activity by reversible means. All these changes are nearly ubiquitous in established cancers.

“If you look at most solid tumors in adults, it looks like someone set off a bomb in the nucleus,” Hahn says. “In most cells, there are big pieces of chromosomes hooked together and duplications or losses of whole chromosomes.” Cancer biologists call such cells aneuploid.

Almost a century ago German biologist Theodor Boveri noticed the strange imbalance in cancer cells between the numbers of maternal versus paternal chromosomes. He even suggested that aneuploid cells might cause the disease. But scientists could find no recurrent pattern to the chromosomal chaos—indeed, the genome of a typical cancer cell is not merely aneuploid but unstable as well, changing every few generations. So Boveri’s idea was dropped as the search for oncogenes started to bear fruit. The aneuploidy and massive genomic instability inside tumor cells were dismissed as side effects of cancer, not prerequisites.

But the oncogene/tumor suppressor gene hypothesis has also failed, despite three decades of effort, to identify a particular set of gene mutations that occurs in every instance of any of the most common and deadly kinds of human cancer. So now, Hahn says, “the question is which comes first: mutations or aneuploidy?”

There are at least four competing answers. The newest and most controversial theory—that cancers often arise from the very stem cells that give healthy organs their growth and healing abilities—is discussed elsewhere in this issue (see “Stem Cells: The Real Culprits in Cancer?” by Michael F. Clarke and Michael W. Becker, on page 40). So we will focus on the three more established ideas. Let us call them the modified dogma, the early instability theory and the all-aneuploidy theory. Encouragingly, all four of these theories seem to be converging as they bend to accommodate new experimental results.

The modified form of the standard dogma revives an idea proposed in 1974 by Lawrence A. Loeb, now at the University of Washington. He and other geneticists have estimated that, on average, random mutation will affect just one gene in any given cell over the course of a lifetime. Something—a carcinogen, reactive oxidants, or perhaps a malfunction in the DNA duplication and repair machinery of the cell—must dramatically accelerate the mutation rate, Loeb argues.

For many years, he suggested that “early during the genesis of cancer there are enormous numbers of random mutations—10,000 to 100,000 per cell,” but he had little evidence to support the idea. In 2006, however, technology advanced to the point that Loeb was able to test his hypothesis by comparing the rate at which a noncoding portion of the p53 gene suffered small mutations in both normal and malignant human cells. Cancerous cells, his test revealed, harbored anywhere from 65 to 475 mutations per 100 million nucleotides, whereas normal cells had four or fewer. That seems to be a far stretch from the 100,000-fold increase in mutation rate that Loeb had anticipated, but it is nonetheless an important discovery that demands explanation.
For decades, the most widely accepted view of how cancer begins has been that mutations to a handful of special genes eliminate tumor suppressor proteins and activate oncoproteins. More recently, three alternative theories have gained currency. One modifies the standard paradigm by postulating a dramatic increase in the accumulation of random mutations throughout the genomes of

**THE GENESIS OF CANCER: FOUR THEORIES**

**STANDARD DOGMA**

1. Carcinogens, such as ultraviolet sunlight and tobacco, directly alter the DNA sequence of cancer-related genes.
2. Mutations in tumor suppressor genes cause growth-inhibiting proteins encoded by the genes to disappear, allowing the cell to survive and continue dividing when it should not.
3. At the same time, mutations to oncogenes cause oncoproteins to become hyperactive, prompting the cell to grow in situations in which it normally would not.

**MODIFIED DOGMA**

1. Something disables one or more genes needed to accurately synthesize or repair the DNA.
2. As the cell divides, random mutations are introduced and go unrepaired, accumulating by the tens of thousands. Eventually the cancer-related genes are hit.

**EARLY INSTABILITY**

1. Something silences one or more "master" genes that are required for cell division.
2. As the chromosomes are duplicated, mistakes occur. Some daughter cells get the wrong number of chromosomes or chromosomes with missing arms or extra segments. The aberrations get worse with each generation.

**ALL-ANEUPLOIDY**

1. A mistake during cell division produces aneuploid cells.
2. As the dosage of genes in the cell changes as chromosome pieces are added or deleted, the misplaced or truncated chromosomes change the relative amounts of thousands of genes. Teams of enzymes that normally cooperate to copy or fix DNA begin to fail. Most aneuploid cells die as a result.
Loeb’s modified dogma thus may add a prologue to the long-accepted life history of cancer. But the most important plot points in that story would still remain the same: mutations to genes that serve to increase the reproductive success of cells. Mangled and ever changing chromosomes are, in this narrative, mere fortuitous by-products.

Unstable from the Outset

Vogelstein of Johns Hopkins and his former co-worker Christoph Lengauer, now at Novartis, proposed an alternative theory in which chromosomal instability can occur early on. The genetic flux then combines forces with natural selection to produce a benign growth that may later be converted to an invasive malignancy and life-threatening metastases.

In their hypothesis, there are several “master” genes whose function is critical for a cell to reproduce correctly. If just one of these genes is disabled, either epigenetically or by mutation, the cell stumbles each time it attempts the carefully choreographed dance of cell division, muddling some of the chromosomes into an aneuploid state. One result is to increase 100,000-fold the rate at which cells randomly lose one of the two alleles of their genes. For a tumor suppressor gene, a lost allele may effectively put the gene out of commission, either because the remaining copy is already mutated or because of the haploinsufficiency effect. Lengauer and Vogelstein still assume that some cancer genes must be altered before a malignancy can erupt.

Some observations do support the early instability theory. In 2000 Lengauer’s laboratory examined colon adenomas—benign polyps that occasionally turn malignant—and observed that more than 90 percent had extra or missing pieces of at least one chromosome. Other researchers have discovered similarly aberrant chromosomes in precancerous growths taken from the stomach, esophagus and breast.

The early instability theory still has some loose ends, however. How can cells...
with shifty chromosomes outcompete their stable counterparts? Under normal conditions, they probably do not, suggests immunologist Jarle Breivik of the University of Oslo. But in a “war zone,” where a carcinogen or other stressor is continually inflicting damage to cells, normal cells stop dividing until they have completed repairs to their DNA. Genetically unstable cells get that way because their DNA repair systems are already broken. So they simply ignore the damage, keep on proliferating, and thus pull ahead, Breivik hypothesizes.

But what jumbles the chromosomes in the first place? No genes have yet been conclusively identified as master genes, although several strong suspects have surfaced. Beth A. A. Weaver of the University of Wisconsin–Madison and her collaborators may have uncovered a clue in their studies of a gene called CENP-E. The protein produced from this gene is one of several that are crucial for guiding the chromosomes as they replicate and separate during cell division. At a meeting of the Society for Chromosomal Cancer Research in Oakland, Calif., this year, Weaver reported that in mice embryos missing one of their two copies of CENP-E (and thus making only half the normal amount of CENP-E protein), chromosomes quickly went askew. Within a couple of weeks, more than half the embryonic cells were aneuploid. And when these animals grew up, they developed many more spleen and lung cancers than did control mice with normal CENP-E genes.

Aneuploidy All the Way Down

On the other hand, maybe cells can become malignant even before any master genes, oncogenes or tumor suppressor genes are mutated. Peter Duesberg of the University of California, Berkeley, has put forth a third theory: nearly all cancer cells are aneuploid (leukemia being one exception) because they start that way. Lots of things can interfere with a dividing cell so that one of its daughter cells is cheated of its normal complement of 46 chromosomes and the other daughter is endowed with a bonus. Asbestos fibers, Duesberg notes, can physically disrupt the process.

Most aneuploid cells are stillborn or growth-retarded. But in the rare survivor, he suggests, the dosage of thousands of genes is altered. That corrupts teams of enzymes that synthesize and maintain DNA. Breaks appear in the double helix, destabilizing the genome further. “The more aneuploid the cell is, the more unstable it is, and the more likely it will produce new combinations of chromosomes that will allow it to grow anywhere,” Duesberg explains.

Unlike the three other theories, the all-aneuploidy hypothesis predicts that the emergence and progress of a tumor are more closely connected to the assortment of chromosomes in its cells than to the mutations in the genes on those chromosomes. Some observations do seem to corroborate the idea.

Thomas Ried, chief of cancer genomics at the National Cancer Institute, has obtained supporting evidence in humans from his investigation of aneuploidy in cervical and colorectal cancers, which he presented at the Oakland conference. “Unequivocally, there are recurrent patterns of genomic imbalances,” Ried avers. “Every single case of [nonhereditary] colorectal cancer, for example, has gains of chromosomes 7, 8, 13 or 20—or a loss of 18. In cervical cancer, aneuploidy of chromosome 3 happens very early, and those cells seem to have a selective advantage.” Ried finds the average number of abnormal chromosomes increasing gradually from 0.2 in a normal cell to 12 in the cells of metastatic colon tumors.

“So I actually think Duesberg is right that aneuploidy can be the first genetic aberration in cancer cells,” Ried says. “But he also argues that no gene mutations are required. This is simply not true.”

Stopping Cancer at Its Roots

Neither the standard dogma nor any of the new theories that challenge it can fully untangle the knotted roots of the 100-odd diseases we call cancer and explain them as variations of a single principle. And all the theories will need to be expanded to incorporate the still mysterious role of epigenetic phenomena, which may be pivotal.

It is important to determine which of the ideas is more right than the others, because they each make different predictions about the kinds of therapy that will succeed best against the most common and lethal cancers. In the standard view, tumors are in effect addicted to the proteins produced by oncogenes and are poisoned by tumor suppressor proteins. Medicines should therefore be designed to break the addiction or supply the poison. Indeed, this strategy is exploited by some newer drugs, such as Gleevec (for rare forms of leukemia and stomach cancer) and Herceptin (for one variety of advanced breast cancer).

But all existing therapies, including Gleevec and Herceptin, fail in some patients because their tumors evolve into a resistant strain. Loeb fears that there may be no easy way around that problem; his latest work, after all, suggests that a tumor of any significant size harbors up to a trillion random mutations. “If I am right, then within any given tumor, there will be cells with random mutations that protect them from any
Hermann J. Muller

1960 Discovery that an exchange of DNA between chromosomes 9 and 22 leads to chronic myelogenous leukemia

1971 Alfred G. Knudson explains different rates of inherited and spontaneous retinal cancer with the hypothesis that two "hits," or damaging mutations, are needed to disable both alleles of the RB gene and that one mutation can be inherited

1974 Lawrence A. Loeb argues that random mutations must accumulate much faster than is normal inside cells that become malignant

1990 Vogelstein and Eric R. Fearon publish a model of sequential gene mutations that lead to colon cancer

1996 Robert A. Weinberg and colleagues isolate RB, the first tumor suppressor gene

1997 Christoph Lengauer, Vogelstein and co-workers demonstrate dramatic increase in gain and loss of chromosomes in colon tumor cells and propose that chromosomal instability is a critical early event that leads to the mutation of oncoproteins and tumor suppressor genes

2002 Thomas Ried identifies recurrent patterns of aneuploidy in human cervical and colon cancer

2008 The number of identified cancer genes, now well over 1,000, continues to grow rapidly

For the elderly—who, after all, are the main victims of cancer—a sufficient delay may be as good as a cure. And even better than slowing the growth of a tumor would be to delay its formation in the first place. If Lengauer, Weaver and others succeed in identifying master genes, then it should also be possible to make drugs that protect or restore their function. The Johns Hopkins group has already licensed some of its cell lines to the pharmaceutical industry to use in drug screening.

Screening of a different kind may be the best approach if the all-aneuploidy theory is correct. There are no known means of selectively killing cells with abnormal chromosomes. But a biopsy that turns up a surfeit of aneuploid cells might warrant careful monitoring or even preventive surgery in certain cases. Duesberg suggests that foods, drugs and chemicals should be tested to identify compounds that cause aneuploidy. And Weaver found that although CENP-E-deficient mouse cells were highly tumorigenic if the cells were moderately aneuploid, cells that were highly aneuploid actually suppressed tumor formation. It may be possible to design drugs that kill cancer cells by exacerbating their aneuploidy.

One day science will produce a definitive answer to the question of what causes cancer. It will probably be a very complicated answer, and it may force us to shift our hope from drugs that cure the disease to medicines that prevent it. Even without a clear understanding of why, doctors have discovered that a daily baby aspirin seems to prevent colon adenomas and to lower the risk of one kind of breast cancer in some adults. The effect is small. But it is a step from chemotherapy toward a better alternative: chemoprevention.

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Stem Cells: The Real Culprits in Cancer?

By Michael F. Clarke and Michael W. Becker

A dark side of stem cells—their potential to turn malignant—is at the root of a handful of cancers and may be the cause of many more. Eliminating the disease could depend on tracking down and destroying these elusive killer cells.

After more than 30 years of declared war on cancer, a few important victories can be claimed, such as 85 percent survival rates for some childhood cancers whose diagnoses once represented a death sentence. In other malignancies, new drugs are able to at least hold the disease at bay, making it a condition with which a patient can live. In 2001, for example, Gleevec was approved for the treatment of chronic myelogenous leukemia (CML). The drug has been a huge clinical success, and many patients are now in remission following treatment with Gleevec. But evidence strongly suggests that these patients are not truly cured, because a reservoir of malignant cells responsible for maintaining the disease has not been eradicated.

Conventional wisdom has long held that any tumor cell remaining in the body could potentially reignite the disease. Current treatments therefore focus on killing the greatest number of cancer cells. Successes with this approach are still very much hit-or-miss, however, and for patients with advanced cases of the most common solid-tumor malignancies, the prognosis remains poor.

Moreover, in CML and a few other cancers it is now clear that only a tiny percentage of tumor cells have the power to produce new cancerous tissue and that targeting these specific cells for destruction may be a far more effective way to eliminate the disease. Because they are the engines driving the growth of new cancer cells and are very probably the origin of the malignancy itself, these cells are called cancer stem cells. But they are also quite literally believed to have once been normal stem cells or their immature offspring that have undergone a malignant transformation.

This idea—that a small population of malignant stem cells can cause cancer—is far from new. Stem cell research is considered to have begun in earnest with studies during the 1950s and 1960s of solid tumors and blood malignancies. Many basic principles of healthy tissue genesis and development were revealed by these observations of what happens when the normal processes derail.

Today the study of stem cells is shedding light on cancer research. Scientists have filled in considerable detail over the past 50 years about mechanisms regulating the behavior of normal stem cells and the cellular progeny to which they give rise. These fresh insights, in turn, have led to the discovery of...
similar hierarchies among cancer cells within a tumor, providing strong support for the theory that rogue stemlike cells are at the root of many cancers. Successfully targeting these cancer stem cells for eradication therefore requires a better understanding of how a good stem cell could go bad in the first place.

**Orderly Conduct**

The human body is a highly compartmentalized system made up of discrete organs and tissues, each performing a function essential to maintaining life. Individual cells that make up these tissues are often short-lived, however. The skin covering your body today is not really the same skin that you had a month ago, because its surface cells have all since sloughed off and been replaced. The lining of the gut turns over every couple of weeks, and the life span of the platelets that help to clot blood is about 10 days.

The mechanism that maintains a constant population of working cells in such tissues is consistent throughout the body and, indeed, is highly conserved among all complex species. It centers on small pools of long-lived stem cells that serve as factories for replenishing supplies of functional cells. This manufacturing process follows tightly regulated and organized steps wherein each generation of a stem cell’s offspring becomes increasingly specialized.

This system is perhaps best exemplified by the hematopoietic family of blood and immune cells. All the functional cells found in the blood and lymph arise from a single common parent known as the hematopoietic stem cell (HSC), which resides in bone marrow. The HSC pool represents less than 0.01 percent of bone marrow cells in adults, yet each of these rare cells gives rise to a larger, intermediately differentiated population of progenitor cells. Those in turn divide and differentiate further through several stages into mature cells responsible for specific tasks, ranging from defending against infection to carrying oxygen to tissues [see box on opposite page]. By the time a cell reaches that final functional stage, it has lost all ability to proliferate or to alter its destiny and is said to be terminally differentiated.

The stem cells themselves meanwhile remain undifferentiated, a state they maintain through their unique capacity for self-renewal: to begin producing new tissues, a stem cell divides in two, but only one of the resulting daughter cells might proceed down a path toward increasing specificity. The other daughter may instead retain the stem cell identity. Numbers in the overall stem cell pool can thus remain constant, whereas the proliferation of intermediate progenitors allows populations of specific hematopoietic cell types to expand rapidly in response to changing needs.

The capacity of stem cells to re-create themselves through self-renewal is their most important defining property. It gives them alone the potential for unlimited life span and future proliferation. In contrast, progenitors have some ability to renew themselves during proliferation, but they are restricted by an internal counting mechanism to a finite number of cell divisions. With increasing differentiation, the ability of the progenitors’ offspring to multiply declines steadily.

The practical significance of these distinctions can be observed when hematopoietic stem cells or their descendants are transplanted. After the bone marrow of a mouse is irradiated to destroy the native hematopoietic system, progenitor cells delivered into the marrow environment can proliferate and restore hematopoiesis temporarily, but after four to eight weeks those cells will die out. A single transplanted hematopoietic stem cell, on the other hand, can restore the entire blood system for the lifetime of the animal.

The hematopoietic system’s organization has been well understood for more than 30 years, but similar cellular hierarchies have recently been identified in other human tissues, including brain, breast, prostate, large and small intestines, and skin. Principles of regulated stem cell behavior are also shared across these tissues, including specific mechanisms for controlling stem cell numbers and for directing decisions about the fates of individual cells. Several genes and the cascades of events triggered by their activity—known as genetic pathways—play key roles in dictating stem cells’ fate and function, for example. Among these are signaling pathways headed by the Bmi-1, Notch, Sonic hedgehog and Wnt genes. Yet most of these genes were first identified not by scientists studying stem cells but by cancer researchers, because their pathways are also involved in the development of malignancies.

Many such similarities between stem cells and cancer cells have been noted. The classical definition of malignancy itself

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**Overview/Cancer Stem Cells**

- Cancer cells are often perceived as all having the same potential to proliferate and expand the disease, but in many types of cancer only a small subset of tumor cells has that power.
- The tumor-generating cells share key traits with stem cells, including an unlimited life span and the ability to generate a diverse range of other cell types, and are therefore considered cancer stem cells.
- These malignant progenitors are believed to spring from regulatory failures in damaged stem cells or their immediate offspring.
- Cancer treatments must target cancer stem cells to eradicate the disease.
includes cancer cells’ apparent capacity to survive and multiply indefinitely, their ability to invade neighboring tissues and to migrate (metastasize) to distant sites in the body. In effect, the usual constraints that tightly control cellular proliferation and identity seem to have been lifted from cancer cells.

Normal stem cells’ power to self-renew already exempts them from the rules limiting life span and proliferation for most cells. Stem cells’ ability to differentiate into a broad range of cell types allows them to form all the different elements of an organ or tissue system. A hallmark of tumors, too, is the heterogeneity of cell types they contain, as though the tumor were a very disorderly version of a whole organ. Hematopoietic stem cells in the blood-forming, or hematopoietic, system illustrate principles governing the activity of stem cells in other tissues as well. A small population of hematopoietic stem cells (HSCs) in the bone marrow is the source of most of the different blood and immune cell types that circulate in the human body. HSCs reside in an environmental niche, surrounded by stromal cells that provide important regulatory signals to the stem cell. When new blood or immune cells are needed, an HSC divides to produce one daughter cell that remains in the niche and retains the long-term HSC identity and another short-lived daughter termed a multipotent progenitor cell (MPP). The MPP, in turn, divides to produce progenitors committed to generating cells in the myeloid (blood) or lymphoid (immune) lineages. As the descendants of progenitors become increasingly specialized, they experience a programmed decline in their ability to proliferate until they stop dividing and are said to be terminally differentiated. Only the stem cell retains unlimited proliferative potential through its ability to renew itself indefinitely by dividing without differentiating.
etonic stem cells have been shown to migrate to distant parts of the body in response to injury signals, as have cancer cells.

In healthy stem cells, strict genetic regulation keeps their potential for unlimited growth and diversification in check. Remove those control mechanisms, and the result would be something that sounds very much like malignancy. These commonalities, along with growing experimental evidence, suggest that failures in stem cell regulation are how many cancers get started, how they perpetuate themselves, and possibly how malignancies can spread.

**Achilles’ Heel**

The presence of stem cells in certain tissues, especially those with high cell turnover such as the gut and the skin, seems to be an overly complicated and inefficient system for replacing damaged or old cells. Would it not appear to make more sense for an organism if every cell could simply proliferate as needed to supply replacements for its injured neighbors? On the surface, perhaps—but that would make every cell in the body a potential cancer cell.

Malignancies are believed to arise when an accumulation of “oncogenic” changes to key genes within a cell leads to the abnormal growth and transformation of that cell. Gene mutations typically happen through a direct insult, such as the cell being exposed to radiation or chemicals, or simply through random error when the gene is improperly copied before cell division. Because the rare stem cells are the only long-lived cells in the organs where most cancers develop, they represent a much smaller potential reservoir for cumulative genetic damage that could eventually lead to cancer. Unfortunately, because stem cells are so long-lived, they also become the most likely repository for such damage.

Indeed, stem cells’ longevity would explain why many cancers develop decades after tissues are subjected to radiation—the initial injury may be only the first in a series of mutations required to transform a healthy cell into a malignant one. In addition to accumulating and preserving these oncogenic scars, a stem cell’s enormous proliferative capacity makes it an ideal target for malignancy. Because nature so strictly regulates self-renewal, a cell population already possessing that ability would need fewer additional mutations for malignant transformation than would cells lacking that capacity.

With these considerations in mind, several possible paths to malignancy become apparent. In one model, mutations occur in the stem cells themselves, and their resulting loss of control over self-renewal decisions produces a pool of stem cells predisposed to malignancy. Subsequent additional oncogenic events that trigger proliferation of the malignant cells into a tumor might happen in the stem cells or in their descendants, the committed progenitor cell population. A second model holds that oncogenic mutations initially occur in stem cells but that the final steps in transformation to cancer happen only in the committed progenitors. This scenario would require the progenitors’ lost self-renewal capacity to be somehow reactivated.

Current evidence supports both models in different cancers. And at least one example exists of both processes playing a role in different stages of the same disease. Chronic myelogenous leukemia is a cancer of the white blood cells caused by the inappropriate fusion of two genes. Insertion of the resulting fused gene will transform a normal hematopoietic stem cell into a leukemia stem cell. Untreated, CML invariably progresses to an acute form known as CML blast crisis. Catriona Jamieson and Irving Weissman, both then at the Stanford University School of Medicine, demonstrated that in patients who progressed to CML blast crisis, the specific additional genetic events responsible for this more virulent version of the disease had conferred the ability to self-renew on certain progenitor cells.

**Steady Pursuit**

Over the past decade, evidence that stem cells could become malignant and that only certain cancer cells shared a variety of traits with stem cells strengthened the idea that the driving force underlying tumor growth might be a subpopulation of stemlike cancer cells. The theory has a longer history, but in the past the technology to prove it was lacking.

By the 1960s a few scientists were already beginning to note that groups of cells within the same tumor differed in their ability to produce new tumor tissue. In 1971 C. H. Park and his colleagues at the University of Toronto showed that within a culture of cells taken from an original, or “primary,” myeloma (a cancer affecting plasma cells in bone marrow), the cells displayed significant differences in their ability to proliferate. At the time, Park’s group could not interpret this phenomenon decisively, because at least two explanations were possible: all the cells might have had the ability to multiply in culture but by chance only some of them did, or else...
The existence of cancer stem cells that drive tumor growth has been established in several blood cancers and a handful of solid-tumor types, but how these malignant stem cells arise is still uncertain. Like a normal stem cell, a cancer stem cell has the ability to self-renew by dividing without differentiating and can therefore potentially give rise to an unlimited number of the abnormal differentiated cells that make up the bulk of a tumor. Those progeny have a finite life span and are not themselves tumorigenic—that is, they cannot generate new cancer cells. The behavior of normal stem cells is tightly controlled by their own genetic programming in concert with signals they receive from their environmental niche. Changes in the way cancer stem cells carrying oncogenic gene mutations respond to niche signaling may therefore play an important role in the cells’ final transition to malignancy (a, b and c). Alternatively, mutations in the stem cell might be preserved in its immature descendants, the progenitor cells, which subsequently undergo further mutations that reactivate the self-renewal capacity normally possessed only by stem cells (d). Evidence of all these possibilities has been observed in different cancers.

**Possible Paths to Cancer**

**EXPANDED NICHE.** Cancer stem cells with oncogenic mutations are held in check by healthy niche signals until a further alteration in the cancer stem cells or in the niche causes the niche to expand. The larger niche allows the malignant stem cells to increase their own population and consequently the number of abnormal cells they generate.

**ALTERNATIVE NICHE.** Oncogenic mutations in cancer stem cells include changes that enable the cells to adapt to a new niche. The cancer stem cells can expand their own numbers and proliferation and possibly invade neighboring tissues or metastasize to distant locations in the body.

**NICHE INDEPENDENCE.** Mutation renders stem cells that are already predisposed to malignancy independent of niche signaling, lifting all normal environmental controls on the cancer stem cells’ self-renewal and proliferation.

**SELF-RENEWAL MUTATION.** Progenitor cells predisposed to malignancy by oncogenic mutations inherited from their parent stem cell undergo further mutation that restores the ability to self-renew. These progenitors thereby gain unlimited life span and tumorigenic capacity and become cancer stem cells.
a hierarchy of cells was present in the tumor and cancer stem cells were giving rise to cells that were nontumorigenic, or incapable of proliferation.

Philip J. Fialkow of the University of Washington had already demonstrated in 1967 that the stem cell model was probably the correct one for leukemia. Using a cell-surface protein marker called G-6-PD, which can identify a cell’s lineage, Fialkow showed that in some women with leukemia, both the tumorigenic cells as well as their more differentiated nontumorigenic progeny had all arisen from the same parent cell.

These early studies were critical in the development of the stem cell model for cancer, but they were still limited by researchers’ inability to isolate and examine different cell populations within a tumor. A key event in stem cell biology, therefore, was the commercial availability, beginning in the 1970s, of an instrument called a flow cytometer, which can automatically sort different living cell populations based on the unique surface markers they bear.

A second crucial event in the evolution of cancer stem cell studies was the advent during the 1990s of conclusive tests for self-renewal. Assays to establish self-renewal in human cells did not exist until Weissman of Stanford and John E. Dick of the University of Toronto developed methods that allowed normal human stem cells to grow in mice. Using flow cytometry and this new mouse model, Dick began in 1994 to publish a series of seminal reports identifying cancer stem cells in leukemia. In 2003 Richard Jones of Johns Hopkins University identified a cancer stem cell population in multiple myeloma.

Earlier the same year our own laboratory group at the University of Michigan at Ann Arbor had published the first evidence of cancer stem cells in solid tumors. By transplanting sorted populations of cells from human breast tumors into mice, we were able to confirm that not all human breast cancer cells have the same capacity to generate new tumor tissue. Only one subpopulation of the cells was able to re-create the original tumor in the new environment. We then compared the phenotype, or physical traits, of those new tumors with that of the patient samples and found that the profile of the new tumors recapitulated the original. This finding indicated that the transplanted tumorigenic cells could both self-renew and give rise to all the different cell populations present in the original tumor, including the nontumorigenic cells.

Our study attested to the presence of a hierarchy of cells within a breast cancer similar to those identified in blood malignancies. Since then, the investigation of cancer stem cell biology has exploded, as labs across the world continue to find similar subpopulations of tumorigenic cells in other forms of cancer. In 2004, for example, the laboratory of Peter Dirks of the University of Toronto identified cells from primary human central nervous system tumors with the capacity to regenerate the entire tumor in mice. Since then, the phenotypes of cancer stem cell populations for colorectal cancer, lung cancer, melanoma, prostate cancer and pancreatic cancer have been reported.

A related area of recent intensive investigation is also providing support for the cancer stem cell model. The signaling environment, or niche, in which tumors reside appears to strongly influence the initiation and maintenance of malignancy. Studies of normal body cells as well as of stem cells have already established the essential role of signals emanating from surrounding tissue and the supportive extracellular matrix in sustaining a given cell’s identity and in directing its behavior. Normal cells removed from their usual context in the body and placed in a dish have a tendency to lose some of their differentiated functional characteristics, for example. Stem cells, in contrast, must be cultured on a medium that provides signals telling them to remain undifferentiated, or they will quickly begin proliferating and differentiating—seemingly as though that is their default programmed behavior, and only the niche signals hold it in check.

In the body, stem cell niches are literal enclaves surrounded by specific cell types, such as stromal cells that form connective tissue in the bone marrow. With a few exceptions, stem cells always remain in their niche and are sometimes physically attached to it by adhesion molecules. Progenitor cells, on the other hand, move away from the niche, often under escort by guardian cells, as they become increasingly differentiated.

The importance of niche signaling in maintaining stem cell biology has already been demonstrated in multiple studies. Early indications are that the cell’s recognition of its niche is essential for the cell’s survival as well as its ability to multiply and divide. A cell cultured in a petri dish can survive but cannot proliferate without the appropriate cues from its niche and is therefore unlikely to contribute to the tumor’s growth.
cells’ undifferentiated state and in keeping them quiescent until they are called on to produce new cells suggests that these local environmental signals could exert similar regulatory control over cancer stem cells. Intriguing experiments have shown, for example, that when transplanted into a new niche, stem cells predisposed to malignancy because of oncogenic mutations will nonetheless fail to produce a tumor. Conversely, normal stem cells transplanted into a tissue environment that has been previously damaged by radiation do give rise to tumors.

Many of the same genetic pathways identified with signaling between stem cells and their niche have been associated with cancer, which also suggests a role for the niche in the final transition to malignancy. For example, if malignant stem cells were being held in check by the niche but the niche was somehow altered and expanded, the malignant stem cell pool would have room to grow as well. Another possibility is that certain oncogenic mutations within cancer stem cells could permit them to adapt to a different niche, again letting them increase their numbers and expand their territory. Still a third alternative is that mutations might allow the cancer stem cells to become independent of niche signals altogether, lifting environmental controls on both self-renewal and proliferation.

Closing In

The implications of a stem cell model of cancer for the way we understand as well as treat malignancies are clear and dramatic. Current therapies take aim against all tumor cells, but our studies and others have shown that only a minor fraction of cancer cells have the ability to reconstitute and perpetuate the malignancy. If traditional therapies shrink a tumor but miss these cells, the cancer is likely to return. Shideng Bao, Jeremy N. Rich and their colleagues at Duke University showed that this was indeed the case for glioblastoma, where the stem cells were resistant to radiation. Treatments that specifically target the cancer stem cells could destroy the engine driving the disease, leaving any remaining nontumorigenic cells to eventually die off on their own.

Circumstantial evidence supporting this approach already exists in medical practice. Following chemotherapy for testicular cancer, for example, a patient’s tumor is examined to assess the effects of treatment. If the tumor contains only mature cells, the cancer usually does not recur and no further treatment is necessary. But if a large number of immature-looking—that is, not fully differentiated—cells are present in the tumor sample, the cancer is likely to return, and standard protocol calls for further chemotherapy. Whether those immature cells are recent offspring that indicate the presence of cancer stem cells remains to be proved, but their association with the disease prognosis is compelling.

Stem cells cannot be identified based solely on their appearance, however, so developing a better understanding of the unique properties of cancer stem cells will first require improved techniques for isolating and studying these rare cells. Once we learn their distinguishing characteristics, we can use that information to target cancer stem cells with tailored treatments. If scientists were to discover the mutation or environmental cue responsible for conferring the ability to self-renew on a particular type of cancer stem cell, for instance, that would be an obvious target for disabling those tumorigenic cells.

Encouraging examples of this strategy’s promise have been demonstrated by Craig T. Jordan and Monica L. Guzman of the University of Rochester. In 2002 they identified unique molecular features of malignant stem cells believed to cause acute myeloid leukemia (AML) and showed that the cancer stem cells could be preferentially targeted by specific drugs. In 2005 they reported their discovery that a compound derived from the feverfew plant induces AML stem cells to commit suicide while leaving normal stem cells unaffected.

Some research groups are hoping to train immune cells or antibodies to recognize and go after cancer stem cells. Tobias Schatton, Markus H. Frank and their co-workers at Children’s Hospital Boston showed that an antibody could inhibit the growth of melanoma stem cells. Yet another idea under investigation is that drugs could be developed to force cancer stem cells to differentiate, which should take away their ability to self-renew.

Most important is that cancer investigators are now on the suspects’ trail. With a combination of approaches, aimed at both targeting genetic pathways unique to the maintenance of cancer stem cells and disrupting the cross talk between tumor cells and their environment, we hope to be able soon to find and arrest the real culprits in cancer.
More than 500 million years ago a set of specialized enzymes and proteins evolved to defend our primitive ancestors against assaults from the outside world. If a microbe breached the shell of some Cambrian-era fauna, the members of this early-vintage immune system would stage a savage but coordinated attack on these interlopers—punching holes in cell walls, spitting out chemical toxins, or simply swallowing and digesting the enemy whole. Once the invaders were dispatched, the immune battalion would start to heal damaged cells, or if the attacked cells were too badly damaged it would put them to rest.

This inflammatory immune response worked so well that many aspects of it have been preserved during the protracted aeons of evolution. We know this to be true because studies have found that we share many of the same immune genes as the lowly fruit fly—and vertebrates and invertebrates diverged from a common ancestor in excess of half a billion years ago.

Immunology researchers have long paid relatively little attention to this thuggish innate immune system, basically thinking of it as a crew of biochemical bouncers that pummel anything able to penetrate the tiniest opening in a living being’s skin or shell. They lavished their attention, instead, on the more advanced adaptive immune system, which can marshal antibodies and other weaponry that identify and then target an intruder with a specificity lacking in the untamed innate system.

In the past 15 years, innate immunity has come into its own. Inflammation, its hallmark characteristic, has gained recognition as an underlying contributor to virtually every chronic disease—a list that, besides obvious culprits such as rheumatoid arthritis and Crohn’s disease, includes diabetes and depression, along with major killers such as heart disease and stroke [see “Common Cause,” on page 53]. The possibility of a link with a third major killer—cancer—has received intensive scrutiny in this decade. “The connection between inflammation and cancer has moved to center stage in the research arena,” notes Robert A. Weinberg of the Massachusetts Institute of Technology’s Whitehead Institute for Biomedical Research, who has highlighted the changing emphasis in a revision of his leading textbook, The Biology of Cancer (Garland Science, 2006).

This transformation recognizes that the immune inflammatory state serves as a key mediator of the middle stages of tumor development. Cancer begins with a series of genetic changes that prompt a group of cells to overreplicate and then invade surrounding tissue, the point at which true malignancy begins. Eventually some tumor cells may break off and establish new growths (metas-
TUMOR DEVELOPMENT progresses in some cancers through the effects of what cancer biologists have labeled a "smoldering" inflammation, in which the tumor recruits immune cells that linger in its surroundings and within the malignant mass.
Multiple Lines of Defense

Comprehension of the link between inflammation and cancer requires knowing how the body reacts to invaders—and how normal healing is then subverted into promoting cancer when the inflammatory state lasts too long. After you step on a nail, the bacteria that invade the sole of your foot receive a welcome from an array of proteins and white blood cells that resemble rejects from central casting for the movie Creepshow 2. Just one example: Some 20 complement proteins, so called because they complement other bodily defense mechanisms, chemically spritz pathogens until the invaders explode into a big protoplasmic mess. While the complement system slimes the area, an assemblage known in immunology textbooks as professional phagocytes—literally “expert eating cells”—goes to work.

Lacking table manners, these Pac-Man-like macrophages and neutrophils proceed to engulf and consume the uninvited guests. Other members of the attack brigade include natural killer cells, mast cells and eosinophils. Healing represents more than launching an offensive against invaders. Blood platelets involved with clotting migrate to the break in the skin from an inner layer infused with blood vessels. Enzymes direct the repair of the extracellular matrix, the protein-based mortar in which the cells are immobilized. A scab forms, the skin grows back and the whole process of inflammation ends. Sometimes, though, inflammation does not stop. Any tissue (not just skin) that is chronically inflamed because of the persistent presence of pathogens, toxins or genetic damage helps to spur illness, from heart disease to cancer.

Beyond this first layer of defense, vertebrates are equipped with additional weaponry. The adaptive system learns an invader’s specific molecular signature and then uses it as a target for killing. Among the protagonists are B cells, which produce antibody molecules able to neutralize pathogens or mark them for destruction, and T cells, which prompt infected cells to kill themselves or secrete chemicals that direct the activities of other immune players.

In recent years a body of evidence has accumulated to show that chronic inflammation can play an important role in the progression of some types of tumors from a premalignant state to full-blown disease. A link between cancer and inflammation has long been suspected. In 1863 the prominent German pathologist Rudolf Virchow noted the presence of so-called lymphoreticular infiltrate (white blood cells) in malignant tissue. As early as 1978 Alberto Mantovani of Humanitas Clinical Institute and the University of Milan had observed that innate immune cells tend to congregate around some tumors. Cancer biologist Harold F. Dvorak of Harvard Medical School remarked in 1986 that tumors are “wounds that do not heal.” The status quo, though, lay elsewhere. Even a decade ago many biologists still hewed to the idea that the immune system serves not only to eliminate pathogens but to ferret out cells that are the abnormal precursors of cancer. But a closer look at the microenvironment surrounding tumors found the unexpected.

Hunting Pigeons

In the late 1990s Frances Balkwill of the Institute of Cancer at Queen Mary, University of London, had been doing research on a cytokine (a hormonelike immune signaling molecule) known as tumor necrosis factor (TNF), which was named for its ability to kill cancer cells when administered directly into a tumor at high levels. But when TNF lingers as a chronic, low-level presence in the tumor, it acts very differently. Balkwill’s lab turned off the TNF gene in mice so that the rodents could not produce the protein: to the researchers’ surprise, the mice did not contract tumors. “That really put us as the cat among pigeons,” she recalls. “All the people who were working on TNF as an anticancer agent were horrified. This cytokine they thought was a treatment for cancer was actually working as an endogenous tumor promoter.”

The ready availability of knockout mice, in which the effects of selectively switching off genes could be tested, helped to highlight the cancer-inflammation link. Coussens and her U.C.S.F. colleagues Douglas Hanahan and Zena Werb reported in 1999 that mice engineered with activated cancer genes but without mast cells (another type of innate immune cell) developed premalignant tissue that did not progress to full malignancy. In 2001 Jeffrey W. Pollard and his co-workers at the Albert Einstein College of Medicine described mice that were genetically engineered to be susceptible to breast cancer tumors but that produced precancerous tissue that did not turn fully malignant unless it enlisted the assistance of macrophages.

The altered picture does not completely

THE PLAYERS

The immune system consists of innate cells, which form a first line of defense against pathogens, and members of the adaptive system, which targets invaders with greater specificity.

INNATE

MACROPHAGE

This immune defender engulfs and consumes pathogen invaders.

mast cell

This cell releases histamine and other chemicals that promote inflammation.

granulocyte

Three cell types with tiny granules in their interior—the neutrophil, eosinophil and basophil—participate in the inflammatory response.

DENDRITIC CELL

It presents antigens—fragments of protein or other molecules from pathogens or cancer cells—to adaptive immune cells, inducing the cells to attack carriers of the displayed antigens.

NATURAL KILLER CELL

This cell destroys the body’s own cells that have become infected with pathogens; it also goes after cancer cells.

ADAPTIVE

B CELL

Antigens stimulate this cell to divide and produce antibodies that neutralize invaders or tag them for killing.

T CELL

A killer T cell destroys an infected cell in which it detects the presence of antigens. Other T cells—such as helper and regulatory types—coordinate the immune response.
overturn the old one. In fact, it reveals that the immune system functions as a double-edged sword. The network of molecules and cells, second in complexity only to the brain, remains a paradox: sometimes it promotes cancer; other times it hinders disease. Some types of innate immune cells, such as natural killer cells, can actually protect against tumor growth. Others may nurture a malignancy only when the microenvironment is “polarized” into an inflammatory state; when not, they may blot it out. Inflammation, moreover, produces tumors in many organs but not all—and its link to blood-borne cancers is not well characterized.

When looking for culprits, researchers have often focused their microscopes on macrophages, which occupy a meaningful spot among the white blood cells in the tumor microenvironment. The macrophages are capable of killing tumor cells or sending out an alarm to T cells of the adaptive immune system that something is amiss. But work by Pollard and other researchers has detailed how macrophages are “reeducated” by cancer cells to do their bidding. They be-

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**CANCER BASICS**

A developing malignancy proceeds in stages—a process that may take years, even decades, to fully evolve.

**INITIATION**

Hereditary mutations or exposure to chemicals or radioactivity results in genetic changes in one or more cells.

**PROMOTION**

Cells in premalignant tissue begin to proliferate, often in the presence of an inflammatory stimulus. Their appearance becomes increasingly abnormal.

**PROGRESSION**

Tumor cells begin to invade surrounding tissue and to spread to the blood and lymph nodes, at which point full malignancy develops. Metastases may establish themselves at distant sites.

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**CANCER HIJACKS WOUND HEALING**

Innate immunity responds to an insult, such as a puncture wound, with a cellular and chemical arsenal. Cancer biologists have recently begun to understand how chronically inflamed premalignant tissue uses many of the same biochemical players to promote cancer development.

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**NORMAL WOUND HEALING**

Cells of the innate immune system, including macrophages, neutrophils and mast cells, converge on bacteria, either consuming them or spraying them with toxins. Some of the innate cells emit chemical signals (cytokines) to coordinate the defensive actions. Meanwhile blood platelets and a protein called fibrin form a clot.

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**WOUND HEALING RUN AMOK**

Chronically inflamed precancerous tissue commandeers many of the same innate cells and signals present in the acute immune response to help the tumor grow and develop a blood supply. Ultimately the tumor starts to invade surrounding tissue, the point at which it becomes fully malignant.
come factories for cytokines and growth factors that nurture tumor development.

Turning the macrophages into traitors begins when tumor cells send out help signals that attract cells that become macrophages once they reach the tumors. Inside the tumors, proliferating cells grow so quickly that they begin to die for lack of oxygen. A combination of hypoxia and messages from the tumor cells initiates a process whereby the newly arrived macrophages assume their bad-boy identity as tumor promoters. Cancer biologists give the name tumor-associated macrophages to these mutineers that congregate in and around the tumor.

Biologists have now been able to follow the inflammation link down to the level of individual signaling molecules, providing harder evidence for a connection to carcinogenesis. For example, nuclear factor-kappa B (NF-κB) is a complex of proteins that acts as a master switch for turning inflammation genes on and for controlling cell death. As biological pathways go, NF-κB’s is world-famous, having been discovered and patented for use in drug development by scientific stars that include Nobelists David Baltimore and Phillip A. Sharp and having subsequently become the object of multimillion-dollar patent litigation.

In 2004 Yinon Ben-Neriah and Eli Pikarsky of the Hebrew University of Jerusalem and their colleagues reported that mice engineered to develop hepatitis (which can cause liver cancer) contracted precancerous lesions that did not progress to full malignancy when NF-κB was curtailed through a genetic alteration or when the proinflammatory TNF signaling molecule was shut off. In the latter group, a neutralizing antibody blocked TNF and prevented it from binding to a receptor on the premalignant liver cells; loss of the receptor prevented the TNF from triggering a molecular cascade that turns on the NF-κB master switch. Blocking NF-κB prompted the precancerous liver cells to initiate apoptosis, or programmed cell death. In a related finding that year, Michael Karin and his collaborators at the University of California, San Diego, found that inhibiting NF-κB in mice engineered to develop colitis, which can lead to colon cancer, also promoted apoptosis. Shutting down the pathway in inflammatory cells, such as macrophages, deterred tumor development as well.

So far the clearest evidence of a link between cancer and inflammation is the data demonstrating that inflammation encourages the conversion of precancerous tissue to full malignancy for many cancers. But the biolog-

Genetic damage is the match that lights the fire of malignancy, and inflammation is the fuel that feeds the flames.
The research suggests that a cytokine produced by inflammatory cells near a prostate tumor induces cancer cells to decrease production of a protein that blocks metastasis. This result, Karin notes, may explain the puzzling observation that cutting into tumors, such as for a prostate biopsy, sometimes seems to encourage metastasis. If he is correct, the inflammation generated by the intervention could be at fault. Around the same time, Pollard’s group reported in Cancer Research on a study in mice that observed that macrophages accompany breast tumor cells in their migration toward blood vessels that then transport them to remote sites.

The innate immune system has received the most attention in explorations of how inflammation might cause cancer. As with infections with free radicals, which can damage DNA. But risk of gastric cancer, and the hepatitis C virus...
nate immunity, the adaptive immune system—the T cells and antibodies produced by B cells that target specific molecules on invading cells—contributes to pathology or may also fight against it. For decades, immunotherapies designed to enhance T cell responses against cancer have been explored, though often with disappointing results [see box on preceding page].

Furthermore, an emerging picture has begun to reveal an intricate cross talk between innate and adaptive immune cells that may participate in the promotion of malignant disease. Researchers working on cancer vaccines may need to take account of these interactions in designing their treatments if they are ever to prove effective. One study showed that ovarian tumors produce a signaling molecule that serves to attract regulatory T cells, a subclass of adaptive immune cells responsible for quieting other T cells.

Meanwhile Coussens and her colleagues at U.C.S.F. found in a 2005 study, published in Cancer Cell, that the removal of antibody-making B cells from mice engineered to be prone to skin cancer prevented the tissue changes and angiogenesis that are prerequisites for disease progression. In their normal role as pathogen fighters, B cell–produced antibodies circulate through the bloodstream and mark viruses and bacteria for destruction by innate immune cells. In response to a signal from precancerous tissue, however, the antibodies induce the innate system to collaborate in cancer development. An open research question is how this process starts. One possibility suggests that a cancer cell may send a message to innate immune cells, perhaps dendritic cells, that then activate B cells. Signaling may involve toll-like receptors, which have emerged as prominent intermediaries in innate immune messaging.

Cancer Blockers

THE RECOGNITION that cancer is more like an organ than just a clump of cells with DNA mutations in cell nuclei may also explain why some of the previous approaches to chemotherapy have met with limited success. “People have taken cells and then transformed them in culture and stuck them into animals,” Pollard says. “They grow as little balls. They do certain things there. But they are not complex tissues, whereas a naturally occurring tumor is a very complex tissue.”

Instead of just killing cancer cells—the goal of current drug therapies and radiation—new approaches may supplement existing drugs by slowing inflammation. Without the involvement of macrophages and other innate cells, the premalignant tissue would remain in check. Cancer could, in essence, become a chronic disease akin to rheumatoid arthritis, another inflammatory condition. “Keep in mind almost no one dies of primary cancer,” says Raymond DuBois, provost of the University of Texas M.D. Anderson Cancer Center and a researcher of anti-inflammatory agents for cancer. “A patient almost always dies from a metastasis.”

A pharmaceutical against chronic inflammation represents a more alluring proposition than massacring malignant cells (and, un-
avoidably, healthy ones), a consequence of existing chemotherapies. Taken alone, such an agent might be benign enough to use every day as a preventive for high-risk patients. Epidemiological and clinical studies have shown some promise for the use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin to stave off the onset of some solid tumors. Investigations continue on more selective blocking of the production of prostaglandins, the regulatory molecules that are curtailed by NSAIDs. In particular, drugs that inhibit production of prostaglandin E₂ may curb inflammation and tumor growth, while avoiding the cardiovascular side effects of drugs such as Vioxx and the gastrointestinal problems of the earlier class of NSAIDs. The anti-inflammatory effects of the ubiquitous statins used to lower cholesterol are also being contemplated.

Some treatment options already exist. The drug Avastin inhibits production of the angiogenesis-promoting VEGF, although oncologists must contend with other molecules in the tumor microenvironment that promote blood vessel growth. Drugs developed for more familiar inflammatory diseases may also fight cancer—and these medicines might be combined into HIV-like drug cocktails that also include angiogenesis inhibitors and cell-killing agents.

Inhibitors of TNF have received approval for treatment of rheumatoid arthritis, Crohn’s disease and other disorders and are now in clinical trials for both solid tumors and blood cancers. The drug Rituxan, a monoclonal antibody that represses B cells in rheumatoid arthritis and B cell lymphoma, might prevent the inflammatory response that fuels formation of solid tumors. Other cytokines and related molecules (IL-6, IL-8 and CCL₂, among others) are also potential targets, as is NF-κB.

Some existing compounds, including NSAIDs and even one found in the spice turmeric, exert at least some of their effects by inhibiting NF-κB. But major pharmaceutical laboratories are investigating highly selective inhibitors of this molecular linchpin, many of them targeted at the enzymes (such as IκB kinase) that regulate NF-κB activity.

### A Chemical Trojan

**One group** is contemplating a radically ambitious treatment, a molecular Trojan horse of sorts. Claire E. Lewis and Munitta Muthana of the University of Sheffield in England and their colleagues have designed a drug delivery scheme that takes advantage of the natural attraction of macrophages to the oxygen-starved areas in tumors. They have engineered macrophages to deliver a therapeutic virus to hypoxic tumor regions, which respond poorly to conventional treatments such as chemotherapy and radiation because of an insufficient blood supply. Once the macrophages arrive in a tumor (grown in culture so far), each one releases thousands of copies of the virus, which then infect the cancer cells, after which a protein in those cells activates the therapeutic gene in each virus. This action then directs synthesis of a cell-killing toxin. “The macrophage is migrating into a site and doing what we want it to do rather than driving tumor development in a normal way,” Lewis says.

The exact outlines of an anti-inflammatory strategy against cancer have yet to be elucidated. Tweaking immune cells that form a defensive barrier against pathogens bears its own risks. “It’s a very complicated issue,” DuBois notes. “If you magically shut down the immune system, you will have problems with opportunistic infections, just like with AIDS.” Use of TNF blockers in other inflammatory disorders has been linked to tuberculosis and other infections, even potentially lymphoma. Moreover, inhibiting the NF-κB pathway can paradoxically promote cancer in some instances. Constraining NF-κB can at times lead to tissue damage and a process of abnormal regeneration of that tissue that can foster cancer.

Still, it seems likely that a new generation of anti-inflammatory agents will join the chemotherapeutic arsenal. Chronic diseases—and their underlying inflammatory conditions—are hallmarks of an aging population. “We’re all a little bit overinflamed,” Pollard observes. Treating the smoldering embers that surround the tumor rather than just mutant cells could make cancer a disease we can live with.

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**Gary Stix is senior writer for Scientific American.**

**More to Explore**

Dendritic cells catch invaders and tell the immune system when and how to respond. Vaccines depend on them, and scientists are even employing the cells to stir up immunity against cancer.

By Jacques Banchereau
Immune System
They are dendritic cells, a class of white blood cells that encompasses some of the least understood but most fascinating actors in the immune system. Over the past decade, researchers have begun to unravel the mysteries of how dendritic cells educate the immune system about what belongs in the body and what is foreign and potentially dangerous. Intriguingly, they have found that dendritic cells initiate and control the overall immune response. For instance, the cells are crucial for establishing immunological “memory,” which is the basis of all vaccines. Indeed, physicians, including those at a number of biotechnology companies, are taking advantage of the role that dendritic cells play in immunization by “vaccinating” cancer patients with dendritic cells loaded with bits of their own tumors to activate their immune system against their cancer. Dendritic cells are also responsible for the phenomenon of immune tolerance, the process through which the immune system learns not to attack other components of the body.

But dendritic cells can have a dark side. The human immunodeficiency virus (HIV) hitchhikes a ride inside dendritic cells to travel to lymph nodes, where it infects and wipes out helper T cells, causing AIDS. And those cells that become active at the wrong time might give rise to autoimmune disorders such as lupus. In these cases, shutting down the activity of dendritic cells could lead to new therapies.

Rare and Precious
dendritic cells are relatively scarce: they constitute only 0.2 percent of white blood cells in the blood and are present in even smaller proportions in tissues such as the skin. In part because of their rarity, their true function eluded scientists for nearly a century after they were first identified in 1868 by German anatomist Paul Langerhans, who mis- took them for nerve endings in the skin.

In 1973 Ralph M. Steinman of the Rockefeller University rediscovered the cells in mouse spleens and recognized that they are part of the immune system. The cells were unusually potent in stimulating immunity in experimental animals. He renamed the cells “dendritic” because of their spiky arms, or dendrites, although the subset of dendritic cells that occur in the epidermis layer of the skin are still commonly called Langerhans cells. This groundbreaking research laid the foundation for all of the progress that we are seeing in dendritic cell science today. In 2007 Steinman received the Albert Lasker Basic Medical Research Award for his pioneering work in rediscovering and characterizing dendritic cells.

For almost 20 years after the cells’ rediscovery, researchers had to go through a painstakingly slow process to isolate them from fresh tissue for study.
But in 1992, when I was at the Schering-Plough Laboratory for Immunology Research in Dardilly, France, my co-workers and I devised methods for growing large amounts of human dendritic cells from bone marrow stem cells in culture dishes in the laboratory. At roughly the same time, Steinman—in collaboration with Kayo Inaba of Kyoto University in Japan and her colleagues—reported that he had invented a technique for culturing dendritic cells from mice.

In 1994 researchers led by Antonio Lanzavecchia, now at the Institute for Research in Biomedicine in Bellinzona, Switzerland, and Gerold Schuler, now at the University of Erlangen-Nuremberg in Germany, found a way to grow the cells from white blood cells called monocytes. Scientists now know that monocytes can be prompted to become either dendritic cells, which turn the immune system on and off, or macrophages, cells that crawl through the body scavenging dead cells and microbes.

The ability to culture dendritic cells offered scientists the opportunity to investigate them in depth for the first time. Some of the initial discoveries expanded the tenuous understanding of how dendritic cells function.

There are several subsets of dendritic cells, which arise from precursors that circulate in the blood and then take up residence in immature form in the skin, mucous membranes, and organs such as the lungs and spleen. Immature dendritic cells are endowed with a wealth of mechanisms for capturing invading microbes: they reel in invaders using suction cup–like receptors on their surfaces, they take microscopic sips of the fluid surrounding them, and they suck in viruses or bacteria by engulfing them in sacks known as vacuoles. Yong-Jun Liu, a former colleague of mine from Schering-Plough who is now at the University of Texas M. D. Anderson Cancer Center, has found that some immature dendritic cells can also zap viruses immediately by secreting a substance called interferon-alpha.

Once they devour foreign objects, the immature cells chop them into fragments (antigens) that can be recognized by the rest of the immune system [see box on next two pages]. The cells use pitchfork-shaped molecules termed the major histocompatibility complex (MHC) to display the antigens on their surfaces. The antigens fit between the tines of the MHC, which comes in two types, class I and class II. The two types vary in shape and in the ways they acquire their antigen cargo while residing inside cells.

Dendritic cells are very efficient at capturing and presenting antigens: they can pick up antigens that occur in only minute concentrations. As they process antigens for presentation, they travel to the spleen through the blood or to lymph nodes through a clear fluid known as lymph. Once at their destinations, the cells complete their maturation and present their antigen-laden MHC molecules to naive helper T cells, those that have never encountered antigens before. Dendritic cells are the only cells that can educate naive helper T cells to recognize an antigen as foreign or dangerous. This unique ability appears to derive from co-stimulatory molecules on their surfaces that can bind to corresponding receptors on the T cells.

Once educated, the helper T cells go on to prompt so-called B cells to produce antibodies that bind to and inactivate the antigen. The dendritic cells and helper cells also activate killer T cells, which can destroy cells infected by microbes. Some of the cells that have been educated by dendritic cells become “memory” cells that remain in the body for years—perhaps decades—to combat the invader in case it ever returns.

Whether the body responds with antibodies or killer cells seems to be determined in part by which subset of dendritic cell conveys the message and which of two types of immune-stimulating substances, called cytokines, the dendritic cells prompt the helper T cells to make. In the case of parasites or some bacterial invaders, type 2 cytokines are best because they arm the immune system with antibodies; type 1 cytokines are better at mustering killer cells to attack cells infected by other kinds of bacteria or by viruses.

If a dendritic cell prompts the wrong
Present in the lungs, skin, gut and lymph nodes, dendritic cells orchestrate the immune response against invaders (here, bacteria entering a cut in the skin).

Dendritic cells bind to helper T cells, killer T cells and—perhaps—B cells. The binding prompts the helper T cells to make substances called cytokines that stimulate killer T cells and cause B cells to begin making antibodies. The antibodies and killer T cells migrate to the cut to fight the infection. Memory cells persist in case the body becomes infected again.
Dendritic cells ingest bacteria and chop them up into bits called antigens. As they exit infected tissues, they mature and display the antigens using molecules called MHC class I and class II.

After traveling to the lymph nodes in a fluid called lymph, dendritic cells activate other cells of the immune system that are capable of recognizing the antigens they carry. The activation readies the immune cells to fight invaders bearing the antigens.
type of cytokine, the body can mount the wrong offense. Generating the appropriate kind of immune response can be a matter of life or death: when exposed to the bacterium that causes leprosy, people who mount a type 1 response develop a mild, tuberculoid form of the disease, whereas those who have a type 2 response can end up with the potentially fatal lepromatous form.

Cancer Killers
activating naive helper T cells is the basis of vaccines for everything from pneumonia to tetanus to influenza. Scientists are now turning the new knowledge of the role that dendritic cells play in immunity against microbes and their toxins into a strategy to fight cancer.

Cancer cells are abnormal and as such are thought to generate molecules that healthy cells do not. If researchers could devise drugs or vaccines that exclusively targeted those aberrant molecules, they could combat cancer more effectively while leaving normal cells and tissues alone—thereby eliminating some of the pernicious side effects of chemotherapy and radiation, such as hair loss, nausea and weakening of the immune system caused by destruction of the bone marrow.

Antigens that occur only on cancerous cells have been hard to find, but researchers have succeeded in isolating several of them, most notably from the skin cancer melanoma. In the early 1990s Thierry Boon of the Ludwig Cancer Institute in Brussels, Steven A. Rosenberg of the National Cancer Institute in Dardilly, France, and their colleagues independently identified melanoma-specific antigens that are currently being targeted in a variety of clinical trials involving humans.

Such trials generally employ vaccines made of dendritic cell precursors that have been isolated from cancer patients and grown in the laboratory together with tumor antigens. During this process, the dendritic cells pick up the antigens, chop them up and present them on their surfaces. When injected back into the patients, the antigen-loaded dendritic cells are expected to ramp up patients’ immune response against their own tumors.

Various researchers—including our own group as well as scientists at several biotechnology companies—are testing this approach against cancers as diverse as melanoma, B cell lymphoma, and tumors of the prostate and colon. There have been glimmers of success. In September 2001, for instance, my co-workers and I, in collaboration with Steinman’s group, reported that 16 of 18 patients with advanced melanoma to whom we gave injections of dendritic cells loaded with melanoma antigens showed signs in laboratory tests of an enhanced immune response to their cancer. What is more, tumor growth was slowed in the nine patients who mounted responses against more than two of the antigens.

Scientists are now working to refine the approach and test it on larger numbers of patients. So far cancer vaccines based on dendritic cells have been tested only in patients with advanced cancer. Although researchers believe that patients with earlier-stage cancers may respond better to the therapy—their immune systems have not yet tried and failed to eradicate their tumor—several potential problems must first be considered.

Some researchers fear that such vaccines might induce patients’ immune systems to attack healthy tissue by mistake. For instance, vitiligo—white patches on the skin caused by the destruction of normal pigment-producing melanocytes—has been observed in melanoma patients who have received the earliest antimalanoma vaccines. (No major adverse events have been reported, however, in more than 1,000 vaccinated patients.) Conversely, the tumors might mutate to “escape” the immune onslaught engendered by a dendritic cell vaccine. Tumor cells could accomplish this evasion by no longer making the antigens the vaccine was designed to stimulate the immune system against. This problem is not unique to dendritic cells, though: the same phenomenon can occur with traditional cancer therapies.

In addition, tailoring a dendritic cell vaccine to fight a particular patient’s tumors might not be economically feasible. But many scientists are working to circumvent the costly and time-consuming steps of isolating cells from patients and manipulating them in the laboratory for reinjection.

One approach involves prompting dendritic cell precursors already present in a person’s body to divide and start orchestrating an immune response against their tumors. While at Immunex in Seattle, David H. Lynch, now at Bainbridge Biopharma Consulting in Bainbridge Island, Wash., and his co-workers discovered a cytokine that causes mice to make more dendritic cells, which eventually induce the animals to reject grafted tumors. Other scientists, including Drew M. Pardoll of Johns Hopkins University, have observed that tumor cells that have been genetically engineered to secrete large amounts of cytokines that activate dendritic cells have the most potential as cancer vaccines.

Another approach, pioneered by Steinman and his Rockefeller colleague Michel C. Nussenzweig, is to selectively target antigens by coupling them to monoclonal antibodies that bind to receptors on the surface of dendritic cells. These receptors need to allow internalization of the antigens and their processing for presentation on both MHC class I and class II antigens. Several such molecules are now under intense scrutiny. Studies in mice have shown that targeting the antigens in the absence of dendritic cell activation results in tolerance induction. In contrast, delivering the antigen together with dendritic cell activators induces immunity, which can be protective.

Jacques Banchereau has directed the Baylor Institute for Immunology Research in Dallas since 1996. The institute aims to manipulate the human immune system to treat cancer as well as infectious and autoimmune diseases. Before 1996 Banchereau led the Schering-Plough Laboratory for Immunology Research in Dardilly, France. He obtained his Ph.D. in biochemistry from the University of Paris and holds many patents on immunological techniques.
Shutting Immunity Down

In the meantime, other scientists are looking at ways to turn off the activity of dendritic cells in instances where they exacerbate disease instead of fighting it. Usually, in a phenomenon known as central tolerance, an organ in the chest called the thymus gets rid of young T cells that happen to recognize the body’s own components as foreign before they have a chance to circulate. Some inevitably slip through, however, so the body has a backup mechanism for restraining their activity.

But this mechanism, termed peripheral tolerance, appears to be broken in patients with autoimmune disorders such as rheumatoid arthritis, type 1 diabetes and systemic lupus erythematosus. In 2001 my colleagues and I reported that dendritic cells from the blood of people with lupus are unnaturally active. Cells from these patients release high amounts of interferon-alpha, an immune-stimulating protein that causes precursors to grow into mature dendritic cells while still in the bloodstream. The mature cells then ingest DNA, which is present in unusual amounts in the blood of people with lupus, and that in turn causes the individual’s immune system to generate antibodies against his or her own DNA. These antibodies result in the life-threatening complications of lupus when they lodge in the kidneys or the walls of blood vessels.

Accordingly, we propose that blocking interferon-alpha might lead to a therapy for lupus by preventing dendritic cell activation. A similar strategy might prevent organ transplant recipients from rejecting their new tissues.

A new treatment for AIDS might also rest on a better understanding of dendritic cells. In 2000 Carl G. Figdor and Yvette van Kooyk, then at the University Medical Center St. Radboud in Nijmegen, the Netherlands, identified a subset of dendritic cells that makes DC-SIGN, a molecule that can bind to the outer coat of HIV. These cells pick up HIV as they regularly prowl the mucous membranes and deep tissues. When they travel to the lymph nodes, they unwittingly deliver the virus to a large concentration of T cells. Drugs that block the interaction between DC-SIGN and HIV might slow the progression of AIDS.

Other infectious diseases—including malaria, measles and cytomegalovirus—also manipulate dendritic cells for their own ends. Red blood cells that have been infected by malaria parasites, for instance, bind to dendritic cells and prevent them from maturing and alerting the immune system to the presence of the invaders. Several groups of researchers are now devising approaches to prevent such microbes from hijacking dendritic cells; some are even seeking to use supercharged dendritic cells to fight the infections.

As we learn more about the molecules that control dendritic cells, we will find ways to harness their therapeutic potential. The increasing number of scientists and pharmaceutical corporations working on dendritic cells portends that we will soon be able to maximize the biological power of these cells to treat and prevent the diseases that plague humankind.

MORE TO EXPLORE


Background information on the immune system and on experimental cancer therapies such as those using dendritic cells can be found on the American Cancer Society’s Web site: www.cancer.org
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

TAMING VESSELS TO TREAT CANCER

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

By Rakesh K. Jain
receive an adequate dose of whatever anticancer
drug was being applied, those cells could survive—
causing the tumor to grow back sooner or later. Per-
haps 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 with-
in 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 in-
sights, we developed approaches to normalizing tu-
mor 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 can-
cer most effectively.

In recent years we have finally been able to start
testing this idea in cancer patients, and the excite-
ment in our lab was overwhelming when we saw the
first clinical evidence of tumors shrinking in re-
sponse 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 malign-
nancy. But what we have already learned about re-

Overview/Controlling Chaos

- 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.
cules, 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 porosity, we discovered that in

**ABNORMAL VESSELS MAKE TROUBLE**

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 FUNCTION**
- Oversize pores in vessel walls leak fluid into interstitial areas (between cells, vessels and other structures)
- High interstitial fluid pressure blocks transport of drugs out of vessels to tumor tissue

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

**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 acidic, hypoxic environments
- Hypoxia causes changes in gene activity that promote tumor cell migration toward healthy tissues

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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; the accumulation of interstitial fluid produces vessels.

The tumor vessels branch erratically, vary in diameter along their lengths and are generally oversize—all features that contribute to irregular blood flow.

The tumor vessels branch erratically, vary in diameter along their lengths and are generally oversize—all features that contribute to irregular blood flow.

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. By 1994 our observations were 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.

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.
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 the late Judah Folkman, my former Harvard colleague, that tumor growth could be halted by starving the mass using antiangiogenic drugs. Indeed, the drug Avastin, approved by the Food and Drug Administration for use in cancer treatment in 2004, is a VEGF-neutralizing antibody originally developed as such an antiangiogenic drug.

In laboratory testing and clinical trials, Avastin...
duced 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 such as 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. Restor-
ing 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 in late 2006. Led by my Massachusetts General Hospital colleagues Tracy Batchelor and Gregory Sorensen, the trial included 30 patients whose brain tumors, known as glioblastomas, had regrown 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

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**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 of the 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.

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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 PlGF (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 pro-growth 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.
TUMOR-BUSTING

A technique called virotherapy harnesses viruses, those banes of humankind, to stop another scourge—cancer

By Dirk M. Nettelbeck, Ronald D. Alvarez and David T. Curiel

Viruses are some of the most insidious creations in nature. They travel light: equipped with just their genetic material packed tightly inside a crystalline case of protein, they latch onto cells, insert their genes, and co-opt the cells’ energy-producing, gene-copying and protein-making machinery, using them to make thousands of copies of themselves. Once formed, the new viruses percolate to the cell surface, pinch off inside minuscule bubbles of cell membrane and drift away, or else they continue reproducing until the cell finally bursts. In any case, they go on to infect and destroy other cells, resulting in diseases from AIDS to the common cold.

Different viruses cause different diseases in part because each virus enters a cell by first attaching to a specific suction cup–like receptor on its surface. Liver cells display one kind of receptor used by one family of viruses, whereas nerve cells display another receptor used by a different viral family, so each type of virus infects a particular variety of cell. Cancer researchers have envied this selectivity for years: if they could only target cancer therapies to tumor cells and avoid damaging normal ones, they might be able to eliminate many of the noxious side effects of cancer treatment.

Some scientists, including ourselves, are now genetically engineering a range of viruses that act as search-and-destroy missiles: selectively infecting and killing cancer cells while leaving healthy ones alone. This new strategy, called virotherapy, has shown promise in animal tests, and clinical trials involving human patients are now under way. Researchers are evaluating virotherapy alone, as well as viruses “armed” with therapeutic genes that enable them to administer traditional chemotherapies solely to tumor cells. They are also developing methods to label viruses, or the cells infected by these viruses, to track the movement of the viral agents in patients.

One of the first inklings that viruses could be useful in combating cancer came in 1912, when an Italian gynecologist observed the regression of cervical cancer in...
A technique called virotherapy harnesses viruses, those banes of humankind, to stop another scourge—cancer. Adenoviruses explode from a cancer cell that has been selectively infected in order to kill it. The viruses can spread to and wipe out other tumor cells.
Overview/Anticancer Viruses

- Virotherapy is a strategy to treat cancer by selectively infecting and killing tumor cells. Researchers are testing various approaches to target viruses to cancer cells, leaving normal cells untouched.
- The viruses used in virotherapy can kill tumor cells either by bursting them open or by combining virus replication with the delivery of genes that make the cells more susceptible to traditional chemotherapies.
- The same types of viruses used in virotherapy can also be labeled with fluorescent tags or devices for detection by nuclear medicine. Once delivered into the body, they home in on cancer cells. In the future, physicians might be able to use this imaging technique to detect the presence of tiny tumors.

TARGETING MELANOMA

The skin cancer melanoma can be highly lethal unless detected early; it arises from pigment cells in the skin or eye, called melanocytes, by uncontrolled growth and spread. Scientists are using the virotherapy approach to selectively kill melanoma cells while leaving healthy cells alone. One technique for studying melanoma involves combining melanoma cells with other types of cells that can be infected by viruses. This allows researchers to test the effectiveness of virotherapy in a controlled environment.

Adenoviruses are distinct from the types of viruses usually used in gene therapy to treat inherited disorders. Gene therapy traditionally employs retroviruses to splice a functioning copy of a gene permanently into the body of a patient in whom that gene has ceased to work properly. Unlike retroviruses, however, adenoviruses do not integrate their DNA into the genes of cells they infect; the genes they ferry into a cell usually work only for a while and then are lost. Scientists have investigated adenoviruses extensively in gene therapy approaches to treat cancer, in which replication-defective derivatives of the viruses are exploited as vectors for transferring into cells genes that, for example, make cancer cells more susceptible than normal ones to chemotherapy. In general, tests involving adenovirus vectors have been safe, but regrettably a volunteer died in 1999 after receiving an infusion of adenoviruses as part of a clinical trial to test a potential gene therapy for a genetic liver disorder [see box on page 78].

Gene therapists have been working to tailor adenoviruses and other viral vectors, or gene-delivery systems, to improve their safety and reduce the chances that such a tragedy might occur again. It is perhaps even more essential for researchers such as ourselves, who are investigating virotherapy, to develop safer, more targeted vectors, because virotherapy by definition aims to kill the cells the viruses infect and thereby produce a new generation of infectious viruses, not just insert a therapeutic gene into them. Replicating in the wrong cells could be dangerous.
viruses have not evolved to infect and kill tumor cells. Unfortunately, adenoviruses bind more efficiently to the variety of normal tissues in the human body than they do to most tumor cells. We can reverse this pattern using specially generated adapter molecules made of antibodies that snap onto the arms of the virus like sockets on a socket wrench. By attaching carefully chosen antibodies or other molecules that selectively bind only to a specific protein found on tumor cells, we can render adenoviruses unable to infect any cells but cancerous ones. Once the antibody-bearing virus latches onto a targeted cell, the hapless cell engulfs it in a membrane sac and pulls it inside. As the sac disintegrates, the viral capsid travels to a pore in the cell’s nucleus and injects its own DNA. Soon the viral DNA directs the cell to make copies of the viral DNA, synthesize viral proteins and combine the two into thousands of new adenoviruses. When the cell is full to capacity, the virus prompts the cell to burst, releasing the new viruses to spread to other cells.

The viruses can also be engineered more directly. In this regard, Curiel’s group at the University of Alabama’s Gene Therapy Center has designed adenoviruses that bind to cellular proteins called integrins. These molecules help cells stick to the network of connective tissue, called the extracellular matrix, that organizes the cells into cohesive tissues. Although integrins are also made by healthy cells, cancer cells produce them in abundance as they become metastatic and begin to squeeze through tissue layers and travel throughout the body. The University of Alabama research group has had encouraging results using the engineered viruses in mice bearing human ovarian cancers. The viruses homed in on the ovarian tumor cells and killed them, ridding the treated animals of the disease.

Transcriptional targeting generally takes advantage of genetic switches (promoters) that dictate how often a given gene is functional (gives rise to the protein it encodes) in a particular type of cell. Although each body cell contains the same encyclopedia of genetic information, some cells use different chapters of the encyclopedia more often than others to fulfill their specialized tasks. Skin cells called melanocytes, for instance, must make much more of the pigment melanin than do liver cells, which have little use for the protein. Accordingly, the promoter for the key enzyme for making melanin gets turned on in melanocytes but generally is off in most other body tissues. In the deadly skin cancer melanoma, the gene encoding this enzyme is frequently fully functional, making the tumors appear black. We, and others, have engineered adenoviruses that have a promoter for the enzyme adjacent to genes that are essential for the viruses’ ability to replicate. Although these viruses might enter normal cells, such as liver cells, they can reproduce only inside melanocytes, which contain the special combination of proteins needed to turn on the promoter.

Researchers are currently tailoring adenoviruses with a variety of promoters that limit their activity to particular organs or tissues. In liver cancers, for example, the promoter for the gene α-fetoprotein—which is normally shut down after fetal development—becomes reactivated. Adenoviruses containing that same promoter hold promise for eradicating liver tumors. Scientists led by Jonathan W. Simons, now at the Prostate Can-
Two main strategies are being explored for virotherapy, which is the technique of using reproducing viruses to kill tumors. In the first method, dubbed transductional targeting (below), scientists are attempting to engineer viruses such as adenoviruses—which normally cause respiratory infections—to selectively infect and destroy only cells that have turned cancerous. They are attaching adapter molecules onto the viral outer coat proteins or directly modifying these proteins to try to prevent the viruses from entering normal cells and to instead prompt them to home in on tumor cells.
The second approach (below) involves placing a snippet of DNA called a tumor-specific promoter next to one of the adenovirus’s essential genes. The promoter acts as an “on” switch that permits the gene to function only in cancer cells. The engineered viruses can enter normal cells, but they cannot reproduce and kill them. Once they enter cancer cells, however, the tumor-specific promoter lets them make thousands of copies of themselves and ultimately burst the cancer cells. They can then spread to—and destroy—other tumors.

—D.M.N., R.D.A. and D.T.C.

**VIROTHERAPY WITH TRANSCRIPTIONAL TARGETING**

Engineered adenovirus with tumor-specific promoter linked to essential virus gene

Infection occurs, but normal cell does not have switch to turn on viral gene; virus cannot replicate or kill cell

Cell bursts, and virus infects and kills other cancer cells

Cancer cell has switch to turn on viral replication genes

Promoter

NORMAL CELL

NORMAL DNA

VIRAL DNA

CELL DNA

CANCER CELL
virotherapists have tested the approach in men whose prostate cancer recurred following treatment with radiation. The researchers used adenoviruses that had been engineered by Cell Genesys to contain the promoter for prostate-specific antigen, a protein made in abundance by prostate tumors. They administered the virotherapy to 20 men who received varying doses of the adenoviruses. In 2001 Simons and his colleagues reported that none of the men experienced serious side effects and that the tumors of the five men who received the highest doses of the virotherapy shrank by at least 50 percent.

**Other Strategies**

Virotherapists might end up combining the transductional and transcriptional targeting strategies to ensure that the viruses kill only tumor cells and not normal ones. Adenoviruses engineered to contain the promoter for the enzyme that makes melanin, for instance, can also replicate in normal melanocytes, so on their own they might cause spots of depigmentation. And adenoviruses that are designed to bind to receptors on the surfaces of tumor cells can still invade a small proportion of healthy cells. But viruses altered to have several fail-safe mechanisms would be expected to be less likely to harm normal cells. There are no results at present, however, to demonstrate that a combination of approaches makes viruses more targeted.

**But Is It Safe?**

Many approaches to virotherapy use adenoviruses, which caused a death in a gene therapy clinical trial.

In September 1999 18-year-old Jesse Gelsinger died after receiving an infusion of adenoviruses into his liver. He had a mild form of an inherited liver disease called ornithine transcarbamylase deficiency (OTCD) and was participating in a clinical trial of a new gene therapy to use adenoviruses to ferry a corrected copy of the gene encoding OTCD into his liver cells. Unfortunately, four days after an infusion of the viruses, he died of acute respiratory distress syndrome and multiple organ failure, apparently caused by an overwhelming immune reaction to the large dose of adenoviruses he had been administered as part of the trial.

Although Gelsinger’s death was part of a gene therapy trial, the tragedy also has ramifications for the young field of virotherapy. Gene therapy uses crippled versions of viruses such as adenoviruses to introduce a new gene into cells; virotherapy employs actively replicating viruses (which may or may not contain added genes), including adenoviruses, to kill specific types of cells.

Gelsinger’s autopsy showed that the engineered adenoviruses had spread to his spleen, lymph nodes and bone marrow, and an examination of his records revealed that his liver function was probably too impaired for him to be a volunteer in the trial. A number of scientists have also suggested that he might have mounted such an extreme immune reaction because he had previously been infected with a naturally occurring adenovirus.

Since Gelsinger’s death, gene therapists and virotherapists alike have focused on refining adenoviruses to make them safer. But researchers are still unsure why Gelsinger reacted so violently to the adenoviral infusions: a second patient participating in the same clinical trial tolerated a similar dose of the viruses. And hundreds of other people worldwide have been treated so far with adenoviruses, including replication-competent adenoviruses, with no serious side effects.

A National Institutes of Health report generated in the aftermath of Gelsinger’s demise recommends that all participants in such clinical trials be monitored closely for toxic reactions before and after the infusion of therapeutic viruses. It also stipulates that volunteers be screened for any predisposing conditions that would increase their sensitivity for the viruses.

—D.M.N., R.D.A. and D.T.C.
Selected Companies Involved in Virotherapy

<table>
<thead>
<tr>
<th>COMPANY</th>
<th>HEADQUARTERS</th>
<th>VIRUS</th>
<th>DISEASES</th>
<th>VIRAL MODIFICATIONS</th>
<th>CLINICAL TRIAL STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BioVex</td>
<td>Abingdon, England</td>
<td>Herpes simplex</td>
<td>Breast cancer and melanoma</td>
<td>Carries the gene for granulocyte-macrophage colony stimulating factor, an immune system stimulant</td>
<td>Phase I/II</td>
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<td></td>
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<td>virus (HSV)</td>
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<tr>
<td>Cell Genesys</td>
<td>South San Francisco,</td>
<td>Adenovirus</td>
<td>Prostate cancer</td>
<td>Targeted to prostate cancer cells using prostate-specific promoters</td>
<td>Phase I/II</td>
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<tr>
<td>Crusade</td>
<td>Glasgow, Scotland</td>
<td>HSV</td>
<td>Glioma (brain cancer), head and neck cancer, melanoma</td>
<td>Has a gene deletion that restricts it to actively dividing cells such as cancers</td>
<td>Phase II for glioma and head and neck cancer; phase I for melanoma</td>
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<tr>
<td>Laboratories</td>
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<tr>
<td>MediGene</td>
<td>Martinsried, Germany</td>
<td>HSV</td>
<td>Glioma and colon cancer that has spread (metastasized)</td>
<td>Harbors two gene deletions that prevent it from reproducing in normal cells</td>
<td>Phase II for glioma; phase I for colon cancer metastases</td>
</tr>
<tr>
<td>Oncolytics</td>
<td>Calgary, Alberta</td>
<td>Reovirus</td>
<td>Prostate cancer and glioma</td>
<td>Able to replicate only in cancer cells bearing the activated oncogene RAS</td>
<td>Phase II for prostate cancer; phase I/II for glioma</td>
</tr>
<tr>
<td>Biotech</td>
<td>Birmingham, Ala.</td>
<td>Adenovirus</td>
<td>Ovarian cancer</td>
<td>Expanded tropism for cancer cells and reduced replication in normal cells</td>
<td>Phase I/II</td>
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<tr>
<td>VectorLogics</td>
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NOTE: Phase I tests are designed to evaluate safety in small numbers of patients. Phases II and III are intended to determine the appropriate dose and efficacy, respectively.

the immunological response, and any antitumor effects of the adenovirus. To date, nine patients have been treated with dosages of up to 10 billion virus particles a day. No toxicity has been detected, and more patients are joining the trial.

Researchers are also arming therapeutic viruses with genes that make the cells they infect uniquely susceptible to chemotherapy. The technique involves splicing into the viruses genes that encode enzymes that turn nontoxic precursors, or “prodrugs,” into noxious chemotherapy agents. In one example, which was reported in 2002, André Lieber of the University of Washington and his co-workers designed adenoviruses to carry genes encoding the enzymes capable of converting innocuous prodrugs into the anticancer compounds camptothecin and 5-fluorouracil. The scientists engineered the viruses so that they could make the enzymes only in actively dividing cells, such as cancer cells. When they injected the viruses and the prodrugs into mice bearing implanted human colon or cervical cancer cells, they found that the viruses reproduced and spread in the tumors.

Such “smart” virotherapies are the vanguard of the future. But physicians will also need to track the activity of virotherapies in a patient’s body to best assess how well the strategies are working and to refine them further. Virotherapists are now teaming with radiologists to establish novel imaging technologies to easily measure how effectively a given virotherapy is replicating.

The imaging strategies involve inserting a gene that governs the production of a tracer molecule into a virus or virus-infected cell. The tracer can be either a fluorescent protein that can be observed directly or one that binds to, or activates and traps, the radionuclides used in standard radiological imaging techniques. The fluorescent protein might work best for cancers that are accessible by an endoscope, such as cancers of the larynx. Physicians could peer into the endoscope and see exactly where the viruses—and, therefore, cancer cells—are by looking for fluorescence. So far the approach has worked best with viruses that do not kill cells, however. Nevertheless, we are convinced that such sophisticated imaging technologies will enable scientists to draw more meaningful conclusions from future clinical trials of virotherapy.

In 1995 gene therapy pioneer W. French Anderson of the University of Southern California School of Medicine predicted in Scientific American that “by 2000 … early versions of injectable vectors that target specific cells will be in clinical trials.” These trials indeed began on schedule, as well as some he could not have envisioned then. We envision a substantial role for viruses—that is, therapeutic viruses—in 21st-century medicine.
Stories of vampires date back thousands of years. Our modern concept stems from Bram Stoker’s quirky classic *Dracula* and Hollywood’s Bela Lugosi—the romantic, sexually charged, bloodsucking outcast with a fatal susceptibility to sunlight and an abhorrence of garlic and crosses. In contrast, vampires of folklore cut a pathetic figure and were also known as the undead. In searching for some underlying truth in vampire stories, researchers have speculated that the tales may have been inspired by real people who suffered from a rare blood disease, porphyria. And in seeking treatments for this disorder, scientists have stumbled on a new way to attack other, more common serious ills.

Porphyria is actually a collection of related diseases in which pigments called porphyrins accumulate in the skin, bones and teeth. Many porphyrins are benign in the dark but are transformed by sunlight into caustic, flesh-eating toxins. Without treatment, the worst forms of the disease (such as congenital erythropoietic porphyria) can be grotesque, ultimately exacting the kind of hideous disfigurement one might expect of the undead. The victims’ ears and nose get eaten away. Their lips and gums erode to reveal red, fanglike teeth. Their skin acquires a patchwork of scars, dense pigmentation and deathly pale hues, reflecting underlying anemia. Because anemia can be treated with blood transfusions, some historians speculate that

By Nick Lane
in the dark ages people with porphyria might have tried drinking blood as a folk remedy. Whatever the truth of this claim, those with congenital erythropoietic porphyria would certainly have learned not to venture outside during the day. They might have learned to avoid garlic, too, for some chemicals in garlic are thought to exacerbate the symptoms of the disease, turning a mild attack into an agonizing reaction.

While struggling to find a cure for porphyria, scientists came to realize that porphyrins could be not just a problem but a tool for medicine. If a porphyrin is injected into diseased tissue, such as a cancerous tumor, it can be activated by light to destroy that tissue. The procedure is known as photodynamic therapy, or PDT, and has grown from an improbable treatment for cancer in the 1970s to a sophisticated and effective weapon against a diverse array of malignancies today and, most recently, for macular degeneration and pathological myopia, common causes of adult blindness. Ongoing research includes pioneering treatments for coronary artery disease, AIDS, autoimmune diseases, transplantation rejection and leukemia.

Molecular Mechanisms

The substances at the heart of porphyria and photodynamic therapy are among the oldest and most important of all biological molecules, because they orchestrate the two most critical energy-generating processes of life: photosynthesis and oxygen respiration. Porphyrins make up a large family of closely related compounds, a colorful set of evolutionary variations on a theme. All porphyrins have in common a flat ring (composed of carbon and nitrogen) with a central hole, which provides space for a metal ion such as iron or magnesium to bind to it. When aligned correctly in the grip of the porphyrin rings, these metal atoms catalyze the most fundamental energy-generating processes in biology. Chlorophyll, the plant pigment that absorbs the energy of sunlight in photosynthetic processes in biology. Chlorophyll, the plant pigment that absorbs the energy of sunlight in photosynthesis, is a porphyrin, as is heme, which is at the heart of the oxygen-transporter protein hemoglobin and of many enzymes vital for life, including cytochrome oxidase (which generates energy by transferring electrons to oxygen in a critical step of cellular respiration).

Porphyria arises because of a flaw in the body’s heme-making machinery. The body produces heme and other porphyrins in a series of eight coordinated stages, each catalyzed by a separate enzyme. Iron is added at the end to make heme. In porphyria, one of the steps does not occur, leading to a backlog of the intermediate compounds produced earlier in the sequence. The body has not evolved to dispose of these intermediates efficiently, so it dumps them, often in the skin. The intermediates do not damage the skin directly, but many of them cause trouble indirectly. Metal-free porphyrins (as well as metalloporphyrins containing metals that do not interact with the porphyrin ring) can become excited when they absorb light at certain wavelengths; their electrons jump into higher-energy orbitals. The molecules can then transmit their excitation to other molecules having the right kind of bonds, especially oxygen, to produce reactive singlet oxygen and other highly reactive and destructive molecules known as free radicals. Metal-free porphyrins, in other words, are not the agents, but rather the brokers, of destruction. They catalyze the production of toxic forms of oxygen.

Photosensitive reactions are not necessarily harmful. Their beneficial effects have been known since ancient times. In particular, some seeds and fruits contain photosensitive chemicals (photosensitizers) called psoralens, which indirectly led scientists to experiment with porphyrins. Psoralens have been used to treat skin conditions in Egypt and India for several thousand years. They were first incorporated into modern medicine by Egyptian dermatologist Abdo Monem El Mofty of Cairo University some 60 years ago, when he began treating patients with vitiligo (a disease that leaves irregular patches of skin without pigment) and, later, those with psoriasis using purified psoralens and sunlight. When activated by light, psoralens react with DNA in proliferating cells to kill them.

Two American dermatologists, the late Aaron B. Lerner of Yale University and the late Thomas B. Fitzpatrick of Harvard University, were struck by the potential of psoralens. In the 1960s they showed that psoralens are activated by ultraviolet (UVA) rays, and the researchers later refined psoralen therapy using an ultraviolet lamp similar to those used in solaria today. Their method became known as PUVA (short for psoralen with UVA) and is now one of the most effective treatments for psoriasis and other skin conditions.

A Way to Kill Cancer Cells?

In the early 1970s the success of PUVA impressed Thomas J. Dougherty of the Roswell Park Cancer Institute in Buffalo, N.Y., leading him to wonder if a variant of it could be effective against cancer. Activated psoralens can kill rogue cells to settle inflammation, but in comparison with porphyrins they are not potent photosensitizers. If psoralens could kill individual cells, could porphyrins perhaps devour whole tumors? His idea was the beginning of true photodynamic
therapy, in which photosensitizers catalyze the production of oxygen free radicals. The concept was built on pioneering work from German physicians Oscar Raab, then a medical student, and Hermann von Tappeiner, his professor. Around the start of the 20th century, Raab and von Tappeiner showed that acridine, when activated by light, reacts with oxygen to kill protozoa such as paramecia. Von Tappeiner had even gone on to treat patients with skin cancer using eosin, a photosensitive component of coal tar, and white light. Dougherty realized, however, that these early forms of photodynamic therapy lacked the raw power of porphyrins. He also drew on two other medically useful properties of porphyrins discovered in the mid-20th century: porphyrins accumulate selectively in cancer cells and are activated by red light, which penetrates more deeply into biological tissues than do shorter wavelengths, such as white light or UVA.

Dougherty injected a mixture of porphyrins into the bloodstream of mice with mammary tumors. He then waited a few days for the porphyrins to build up in the tumors before shining red light on them. His early setup was primitive, passing light from an old slide projector through a 35-millimeter slide colored red. His results were nonetheless spectacular. The light activated the porphyrins within the tumor, which transferred their energy to oxygen in cells to damage the surrounding tissues. In almost every case, the tumors blackened and died after the light treatment. There were no signs of recurrence.

Dougherty and his colleagues published their data in 1975 in the *Journal of the National Cancer Institute*, with the brave title “Photoradiation Therapy II: Cure of Animal Tumors with Hematoporphyrin and Light.” Over the next few years they refined their technique by using a low-power laser to focus red light on them. They went on to treat more than 100 patients in this way, including people with cancers of the breast, lung, prostate and skin. Their outcomes were gratifying, with a “complete or partial response” in 111 of 113 tumors.

Sadly, though, cancer is not so easily beaten. As more physicians started trying their hand with PDT, some serious drawbacks began to emerge. The affinity of porphyrins for tumors turned out to be a bit of an illusion—porphyrins are taken up by any rapidly proliferating tissue, including the skin, leading to photosensitivity. Although Dougherty’s original patients were no doubt careful to avoid the sun, nearly 40 percent of them reported burns and skin rashes in the weeks after PDT.

Potency was another issue. The early porphyrin preparations were mixtures, and they were seldom strong enough to kill the entire tumor. Some porphyrins are not efficient at passing energy to oxygen; others are activated only by light that cannot penetrate more than a few millimeters into the tumor. Some biological pigments normally present in tissues, such as hemoglobin and melanin, also absorb light and in doing so can prevent a porphyrin from being activated. Even the porphyrin itself can cause this problem if it accumulates to such high levels that it absorbs all the light in the superficial layers of the tumor, thus preventing penetration into the deeper layers.

Many of these difficulties could not be resolved without the help of specialists from other disciplines. Chemists were needed to create new, synthetic porphyrins, ones that had greater selectivity for tumors and greater potency and that would be activated by wavelengths of light able to reach farther into tissues and tumors. (For each porphyrin, light activation and absorption occur only at particular wavelengths, so the trick is to design a porphyrin that has its absorption maximum at a wavelength that penetrates into biological tissues.) Physicists were needed to design sources that could produce light of particular wavelengths to activate the new porphyrins or that could be attached to fine endoscopes and catheters or even implanted in tissues. Pharmacologists were needed to devise ways of reducing the time that porphyrins spent circulating in the bloodstream, thereby restricting photosensitive side effects. Finally, clinicians were needed to design trials that could
Doctors who administer photodynamic therapy deliver photosensitive chemicals called porphyrins intravenously. These chemicals then collect in rapidly proliferating cells and, when exposed to light, initiate a cascade of molecular reactions that can destroy those cells and the tissues they compose. Some targets for the therapy include abnormal blood vessels in the retinas of people with age-related macular degeneration (the leading cause of adult blindness), cancerous tumors, and atherosclerotic plaques in coronary arteries.

**How Photodynamic Therapy Works**

**... AT THE MOLECULAR LEVEL**

1. A porphyrin absorbs light, becoming activated.
2. The activated porphyrin passes this light energy to oxygen molecules, converting them to singlet oxygen.
3. Singlet oxygen reacts with other substances in cells to produce destructive oxygen free radicals; then cells die.

**... IN THE EYE**

1. To treat macular degeneration, a porphyrin (green) is injected into a patient’s arm. It takes just 15 minutes for the porphyrin to accumulate in abnormal blood vessels under the macula, the central part of the retina responsible for color vision.
2. A red laser light activates the porphyrin, which leads to the destruction of the vascular tissue.
3. After therapy halts damage to the retina, the treated vascular tissue is reabsorbed by the body, and the overlying photoreceptors may settle back into place. Because vessel growth could recur, the patient may require several additional treatments.
prove an effect and determine the best treatment regimens.

The ideal drug would be not only potent and highly selective for tumors but also broken down quickly into harmless compounds and excreted from the body. The first commercial preparation, porfimer sodium (Photofrin), was approved by the U.S. Food and Drug Administration for the treatment of various cancers. Although it has been helpful against certain cancers (including esophageal, bladder, head and neck, and skin cancers and some stages of lung cancer), it has not been the breakthrough that had been hoped for and cannot yet be considered an integral part of cancer therapy. Surprisingly, though, the first photosensitizing drug to fulfill most of the stringent criteria for potency and efficacy without causing photosensitivity, verte porfin (Visudyne), was approved in April 2000 by the FDA not to treat cancer at all but to prevent blindness. As the theories converged with reality, researchers came to realize that PDT can do far more than destroy tumors.

Battling Blindness

**ONE THING IT COULD DO,** for instance, was combat age-related macular degeneration (AMD), the most common cause of legal blindness in our maturing Western population. Most people who acquire AMD have a benign form and do not lose their sight, but about a tenth have a much more aggressive type called wet AMD. In this case, abnormal, leaking blood vessels, like miniature knots of varicose veins, grow underneath the retina and ultimately damage the sharp central vision required for reading and driving. As the disease progresses, central vision is obliterated, making it impossible to recognize people’s faces or the details of objects.

Most attempts to hinder this grimly inexorable process have failed. Dietary antioxidants may be able to delay the onset of the disorder but have little effect on the progression of established disease. Until recently, the only treatment proved to slow the progression of wet AMD was a technique called laser photocoagulation. The procedure involves applying a thermal laser to the blood vessels to fuse them and thus halt their growth. Unfortunately, the laser also burns the normal retina and so destroys a small region to prevent later loss of vision in the rest of the eye. Whether this is worth it depends on the area of the retina that needs to be treated. For most people diagnosed with wet AMD, the area is located below the critical central part of vision or is already too large to benefit from laser coagulation.

Against this depressing backdrop, researchers at Harvard and at the biotechnology firm QLT in Vancouver, B.C., reasoned that PDT might halt the growth of these blood vessels and delay or even prevent blindness. If porphyrins could accumulate in any rapidly proliferating tissue—the very problem in cancer—then perhaps they could also accumulate in the blood vessels growing under the retina. Verteporfin, a novel synthetic porphyrin, seemed promising because it had a good track record in preclinical animal studies at QLT and at the University of British Columbia in the late 1980s and early 1990s.

Verteporfin accumulates in abnormal retinal vessels re-
tested to treat coronary artery disease. The new idea is called photoangioplasty, which is now being pursued in clinical trials. It involves injecting a porphyrin into the bloodstream, and then illuminating the artery from within, using a tiny light source attached to the end of a catheter. The light activates the porphyrins in the plaques, destroying the abnormal tissues while sparing the normal walls of the artery.

Coronary angioplasty is a minimally invasive procedure for treating arteries affected by atherosclerosis. It uses a tiny balloon to open arteries, so that atherosclerotic plaques do not occlude the entire vessel. Photoangioplasty could sidestep many of the problems of conventional angioplasty, notably the restenosis (renarrowing) of treated arteries. The procedure involves injecting a porphyrin into the bloodstream, waiting for it to build up in the damaged arterial walls and then illuminating the artery from the inside, using a tiny light source attached to the end of a catheter. The light activates the porphyrins in the plaques, destroying the abnormal tissues while sparing the normal walls of the artery.

An even more ingenious method for treating deep cancers is the use of self-lighting nanoparticles. Wei Chen of the University of Texas at Arlington recently developed a method of attaching porphyrins to “scintillation luminescence nanoparticles.” These nanoparticles generate visible light when exposed to x-rays during radiotherapy, and in turn activates the porphyrin. The method effectively combines radiotherapy with PDT, optimizing both methods and allowing the treatment of large or deeply buried tumors with relatively low-dose radiotherapy. But how will the bulky nanoparticles be targeted at tumor cells? In January, Chen and his colleagues reported preliminary findings suggesting that the entire complex can be attached to folic acid, which is then taken up by folate receptors on cancer cells.

An alternative approach for targeting porphyrins or even nanoparticle complexes at tumors involves attaching them to antibody fragments that recognize cancer cells. Mahendra Deonarain and his colleagues at Imperial College London, who are pioneers of this method, have managed to attach more than 10 porphyrin molecules to a single antibody fragment without destroying its ability to target cancer cells. The group used antibody fragments rather than whole antibodies to reduce the size of the complex and enable it to be cleared from the body more quickly. The team’s initial findings, reported in March, showed the complexes accumulating in tumors at 10 times their concentration in blood and 50 times their concentration in muscle.

Accumulation of porphyrins in active and proliferating cells raises the possibility of treating other conditions in which abnormal cell activation or proliferation plays a role—among them, infectious diseases. Attempts to treat infections with the pigments had long been frustrated by a limited effect on gram-negative bacteria, which have a complex cell wall that obstructs the uptake of porphyrins into these organisms. One solution, developed by Michael R. Hamblin and his colleagues at Harvard, involved attaching a polymer—usually polylysine, a repetitive chain of the amino acid lysine—to the porphyrin. The polymer disrupts the lipid structure of the bacterial cell wall, enabling the porphyrins to gain entry to the cell. Once inside, they can be activated by light to kill the bacteria. In studies of animals with oral infections and infected wounds, the altered porphyrin showed potent antimicrobial activity against a broad spectrum of gram-negative and gram-positive bacteria.

**Other Treatment Avenues**

The success of ophthalmic PDT has inspired research activity in other fields but also reveals the drawbacks of the treatment. In particular, even red light penetrates no more than a few centimeters into biological tissues [see illustration above]. This limitation threatens the utility of PDT in internal medicine—its significance might seem to be skin deep. There are ways of turning PDT inward, however. One ingenious idea is called photoangioplasty, which is now being tested to treat coronary artery disease.
Photodynamic Therapies

The light-activated drugs listed below are a sampling of those on the market or in development.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>TARGET</th>
<th>MAKER</th>
<th>STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEVULAN (5-aminolevulinic acid)</td>
<td>Acne and actinic keratosis (precancerous skin disorder), Barrett’s esophagus (precancerous condition)</td>
<td>DUSA PHARMACEUTICALS Toronto</td>
<td>On the market for actinic keratosis; phase II trials [relatively small studies in humans] have begun for acne</td>
</tr>
<tr>
<td>PHOTOFRIN (porfimer sodium)</td>
<td>Cancers of the esophagus and lung, high-grade dysplasia from Barrett’s esophagus</td>
<td>AXCAN SCANDIPHARM Birmingham, Ala.</td>
<td>On the market for esophageal cancer and nonsmall cell lung cancer; FDA-approved for high-grade dysplasia</td>
</tr>
<tr>
<td>VISUDYE (verteporfin)</td>
<td>Age-related macular degeneration, pathological myopia and ocular histoplasmosis [eye disorders]</td>
<td>OLT and NOVARTIS OPHTHALMICS Vancouver, B.C., and Duluth, Ga.</td>
<td>On the market</td>
</tr>
<tr>
<td>METVIX (methylaminolevulinic acid)</td>
<td>Actinic keratosis, basal cell skin cancer and squamous cell skin cancer</td>
<td>PHOTOCURE Oslo, Norway</td>
<td>FDA-approved for actinic keratosis and basal cell skin cancer</td>
</tr>
<tr>
<td>LS11 (talaporfin sodium)</td>
<td>Colorectal cancer, hepatocellular carcinoma, and glioma</td>
<td>LIGHT SCIENCES ONCOLOGY Bellevue, Wash.</td>
<td>In phase III trials for colorectal cancer with recurrent liver metastases and for hepatocellular carcinoma; phase II trial for glioma completed in February</td>
</tr>
<tr>
<td>TOO_KAD (vascular-targeted PDT)</td>
<td>Prostate cancer</td>
<td>STEBA BIOTECH Toussus-le-Noble, France</td>
<td>In phase II/III trials for prostate cancer after radiation therapy</td>
</tr>
</tbody>
</table>

bacteria. As antibiotic resistance becomes more intractable, targeted antimicrobial PDT could become a useful weapon in the medical arsenal.

Several other, related photodynamic methods hinge on the finding that activated immune cells take up greater amounts of photosensitizing drugs than do quiescent immune cells and red blood cells, sparing the quiet cells from irreversible damage. In most infections, nobody would wish to destroy activated immune cells: they are, after all, responsible for the body’s riposte to the infection. In these cases, targeting immune cells would be equivalent to “friendly fire” and would give the infection free rein to pillage the body.

In AIDS, however, the reverse is true. The AIDS virus, HIV, infects the immune cells themselves. Targeting infected immune cells would then be more like eliminating double agents. In the laboratory, HIV-infected immune cells take up porphyrins, thereby becoming vulnerable to light treatment. In patients, the light could be applied either by withdrawing blood, illuminating it and transfusing it back into the body (extracorporeal phototherapy) or by shining red light onto the skin, in what is called transdermal phototherapy. In the transdermal approach, light would eliminate activated immune cells in the circulation as they passed through the skin. Whether the technique will be potent enough to eliminate diseased immune cells in HIV-infected patients remains an open question.

Autoimmune diseases, rejection of organ transplants, and leukemias are also all linked by the common thread of activated and proliferating immune cells. In autoimmune diseases, components of our own body erroneously activate immune cells. These activated clones then proliferate in an effort to destroy the perceived threat—say, the myelin sheath in multiple sclerosis or the collagen in rheumatoid arthritis. When organs are implanted, activated immune cells may multiply to reject the foreign tissue—the transplanted organ or even the body tissues of the new host, in the case of bone marrow transplants. In leukemia, immune cells and their precursors in the bone marrow produce large numbers of nonfunctional cells. In each instance, PDT could potentially eliminate the unwanted immune cells, while preserving the quiescent cells, to maintain a normal immune response to infection. As in HIV infection, the procedure might work either extracorporeally or transdermally. Much of this research is in late-stage preclinical or early clinical trials. For all the cleverness in exploring possible medical applications, though, we can only hope that more extensive clinical studies will bear fruit.
Breast cancer is the most commonly diagnosed malignancy among women and, after lung cancer, the second leading cause of cancer-related deaths in North America. Yet unlike the survival rate for individuals diagnosed with lung cancer, the rate for women diagnosed with breast cancer has been rising dramatically over the past decade—to the point where breast cancer could soon lose its ranking as the second-greatest cancer killer. Nothing would delight clinicians like us more.

This improvement in overall outlook for women diagnosed with breast cancer is attributable in part to earlier detection, which results from greater awareness of, and access to, regular breast screening. But breast cancer patients are also benefiting from accelerated research that has led to a much better understanding of the disease and a wider variety of treatment choices that doctors can mix and match to tailor therapy for a particular patient. In just the past decade, it has even become possible to target drugs to specific molecules within tumors that help to drive the disease.

Breast cancer was, in fact, the first type of solid-tumor cancer to be treated with this molecular-targeting therapeutic approach, when the drug trastuzumab (Herceptin) was approved in 1998. The protein that trastuzumab was designed to attack, called HER2, promotes aggressive tumor growth. Before trastuzumab, diagnosis with a tumor that overproduces HER2 was dreaded news for patients. Now it can be one of the tumor types with the best prognosis, because doctors have an increasing number of effective weapons against HER2.

The next decade promises to be an exciting and productive time in the field of molecular-targeted cancer therapy: additional drugs currently being tested in people and animals are making it possible to go after an increasing variety of molecular tumor features that play a critical role in the initiation and survival of malignancies and in the cancers’ progression to increasingly threatening stages. Along with improvements in older therapies and supportive care, this newer generation of drugs gives doctors more options for customizing treatment to cope with a tumor’s particular suite of molecular characteristics and reflects our growing realization that breast cancer is not a single disease.

Evolving Treatment Approaches although the prospect of tailoring treatment to the molecular features of individual tumors is incredibly encouraging, prior advances are also contributing to the declining mortality rate for women diagnosed with breast cancer. Improved screening techniques, for instance, are definitely helping to catch and confirm more cases at an earlier stage, which is a boon, because breast cancer is highly curable if detected early. Newer imaging methods include digital mammography (which produces a clearer picture than screen-film mammography), ultrasound and magnetic resonance imaging (MRI).

In addition, surgical approaches to tumor excision have changed over the past 20 years from radical tissue removal in women whose tumor appears confined to a small part of the breast to breast-conserv
FAST FACTS

Inherited mutations in the BRCA1 gene can multiply lifetime breast cancer risk by 10 times, but only in the past year have researchers discovered why. BRCA1 is involved in DNA repair, so its malfunction makes errors in other cancer-promoting genes more likely.

Following a 2002 report that hormone replacement therapy (HRT) increased breast cancer risk in postmenopausal women, HRT use fell. The next year there was a dramatic drop in the incidence of both invasive (7.3 percent) and noninvasive (5.5 percent) breast cancers in the U.S.

In addition to supportive care that increases their quality of life.

Another mainstay of breast cancer treatment, at least for patients with tumors that are determined to be dependent on estrogen or progesterone, is endocrine therapy. Indeed, hormonal manipulations to treat breast cancer date as far back as the 1890s, when doctors observed tumors regressing after they had removed the ovaries of premenopausal women with advanced breast disease. In 1966 researchers identified hormone receptors—molecules that bind to specific hormones—in various tissues, including that of the breast. Subsequent studies showed that a significant number of invasive breast cancers—as many as 75 percent—contain estrogen receptors or progesterone receptors, or both, causing these molecules to quickly become therapeutic targets.

The antiestrogen drug tamoxifen was first approved in the U.S. in 1977 to treat advanced breast cancer in postmenopausal women. The drug molecule binds to the estrogen receptor, preventing estrogen from doing so. Tamoxifen has since proved effective for patients with localized breast tumors that display estrogen or progesterone receptors and as a preventive therapy in healthy women who are at high risk for breast cancer. Meanwhile newer drugs that inhibit the aromatase enzyme, suppressing natural estrogen manufacture in the body, have proved superior to tamoxifen in postmenopausal women.

In a sense, then, estrogen and progesterone receptors became the first molecular features of tumors that could be directly targeted by drugs, although an important distinction should be noted between these targets and newer ones identified in the past decade. The sex steroid receptors promote cell proliferation, or growth, in healthy tissues as well as in tumors, so suppressing their ability to transmit growth signals does help to check tumor

RAISING AWARENESS of the importance of early detection, as well as raising funding for research, has paid off in notable declines in breast cancer mortality in the developed world.
enlargement. And changes to the receptors’ shape or function may sometimes contribute to the general malignant characteristics of tumor cells. But the gene encoding the estrogen receptor is rarely mutated in breast cancer, which means that it is not a true cancer-causing gene.

Perhaps the most important realization in cancer research since the era when sex hormone receptors were discovered is that particular genes, when they become mutated, can cause a normal cell to turn cancerous. Such genes, once they do mutate, are referred to as oncogenes, and they are believed to be responsible both for initiating the transformation of a normal cell into a cancerous one and for driving tumor growth. That is why breast cancer (like all cancers) is described today as fundamentally a disease of genes. An oncogenic mutation, such as a small change in the DNA nucleotide sequence of a gene, might disable a protective gene or boost the activity of a tumor-promoting one. In some cases, entire genes are deleted or duplicated [see sidebar on page 93].

Tumors can now be classified according to the genes that are overactive or suppressed in their cells and according to the resulting changes in the manufacture and function of proteins encoded by those genes. The damaged genes can vary from tumor to tumor, and this heterogeneity at the genetic level explains why breast cancers in individual patients might behave differently. Some cancers have limited invasiveness and metastatic potential, for instance, whereas others spread quickly to distant organs. Knowing the molecular profile of a patient’s tumor should permit a doctor to focus on inhibiting the mechanisms driving that particular tumor, one day choosing from an arsenal of drugs a set that will interfere with the specific molecules involved in the initiation, growth, and spread of the cancer. The success of trastuzumab and other HER2-targeted therapies illustrates the potential of this approach in combating breast cancer.

**Targeting HER2**

In the early 1980s the gene that gives rise to HER2 was first discovered in mutated form in rat neural tumors by investigators at the Massachusetts Institute of Technology, who named that oncogene Neu. Soon researchers realized that the gene was a mammalian version of one previously identified in viruses called ERBB, so Neu also came to be known as ERBB2. This gene was not done accumulating names, however. When scientists identified the protein encoded by ERBB2, they realized that it was closely related to a cell-membrane protein called epidermal growth factor receptor (EGFR). Thus, when they finally isolated the human version of the ERBB2 gene, they named it human epidermal growth factor receptor 2 (HER2).

As it turns out, the entire EGFR family of proteins has proved important to tumor cell growth in a variety of cancers. When activated by specific molecules that bind to them (their ligands), such receptors transmit a proliferation signal to the cell by initiating a cascade of internal molecular interactions—spurring activity by genes whose encoded proteins regulate the activity of still more “downstream” genes. Shortly after the HER2 gene was discovered, scientists noted that it was frequently duplicated in breast cancer cells and that having multiple copies of the gene was associated with a poor prognosis.

Laboratory studies confirmed that adding copies of the HER2 gene to a normal cell could trans-
form it into a cancer cell—a hallmark ability of oncogenes. Because 20 percent of breast cancer tumors overproduce the HER2 protein, it became a therapeutic target for drug researchers. Genentech scientists created trastuzumab in the late 1980s by manufacturing so-called monoclonal antibodies that bind to the HER2 receptor, preventing it from being activated. In clinical trials, it was found that trastuzumab could lengthen the survival of patients with both early-stage and metastatic breast cancer.

The success of trastuzumab has led to the development of similar antibody-based therapies, such as pertuzumab, which binds to HER2 at a different site than trastuzumab and has the added effect of preventing the receptor from interacting with other members of its family in the cell membrane, such as EGFR and HER3. Blocking such interactions reduces growth signaling along the intracellular pathways of molecular communication downstream of these receptors. Pertuzumab can even disrupt certain types of HER2 activation in tumor cells that have become resistant to trastuzumab. Moreover, we have shown that combining trastuzumab with pertuzumab can boost the rate of cell death in breast cancer cells overproducing HER2.

Still another method of wielding antibodies against the HER2 receptor is to attach a potent toxin to them, which the antibodies then transport into the cancer cell. After the toxin-antibody pair is internalized by a cell, the toxin detaches and kills the cell. This approach has been successful in other types of cancer, such as acute myeloid leukemia, and clinical trials are under way in patients with metastatic breast cancer to determine the safety and efficacy of such trastuzumab-based conjugates.

To send a growth signal into a cell, the intracellular region of the EGFR family of proteins must first be acted on by tyrosine kinases, enzymes that chemically modify a segment known as the tyrosine kinase domain. Tyrosine kinases can thus act as growth-stimulating factors, and inhibiting them directly is another way of squelching EGFR-mediated growth signaling in cells. That is why pharmaceutical companies are avidly pursuing the clinical development of such drugs. Lapatinib (Tykerb) is a dual EGFR/HER2 tyrosine kinase inhibitor that has shown remarkable laboratory results, leading to growth arrest and cell suicide in breast cancer cell lines that overproduce HER2.

One way to improve the effectiveness of HER2-targeted therapy is, therefore, to combine a drug such as trastuzumab with a tyrosine kinase inhibitor such as lapatinib. In breast cancer cell lines, that combination produces greater synergistic growth inhibition and higher rates of cell suicide. Even in cell lines that have developed resistance to trastuzumab after long-term treatment, lapatinib has proved just as effective at inducing cell suicide. A recent large (phase III) clinical trial among patients with HER2-overproducing metastatic breast cancer, whose disease had become resistant to trastuzumab, demonstrated that lapatinib plus capecitabine chemotherapy doubled the median time to progression as compared with capecitabine alone. On the basis of these results, in 2007 the U.S. Food and Drug Administration approved the use of lapatinib combined with

![Tumor Pathways Diagram]
capecitabine for treating metastatic disease. Clinical trials to determine lapatinib’s value as an adjuvant treatment in a wider variety of circumstances are ongoing, as are trials of several other tyrosine kinase inhibitors that target HER2 and EGFR.

Finding alternative means of interfering with the same growth pathways is important because, as can happen with trastuzumab, cancer cells often do eventually find ways to evade individual drugs. Also under way is research into how and why cancer cells develop resistance to trastuzumab, so that investigators can use those insights as a guide to designing more effective combinations or new agents for patients whose tumors overproduce HER2.

In studies of cell cultures and animals, for instance, our laboratory has discovered that cancer cells employ many different mechanisms to survive in the presence of trastuzumab, including increasing their production of other growth factor receptors, either from the EGFR/HER family or from other families, such as the insulinlike growth factor 1 (IGF-1) receptor. The cells may also lose or inactivate the tumor suppressor gene PTEN. This gene generally blocks a survival pathway involving the enzyme phosphotidylinositol 3-kinase (PI3K), which allows damaged cells to ignore signals telling them to commit suicide. We have even seen cells lose or disable the extracellular binding site that trastuzumab attaches to on the HER2 receptor.

In light of these observations, identifying additional molecular targets to attack in cells that overproduce HER2, as well as targets in the other 80 percent of tumors that do not have HER2 mutations, is a high research priority.

Expanding the Arsenal

Among the most promising new targets for breast cancer therapy is the IGF-1 receptor as well as the growth hormone molecules that activate it, IGF-1 and IGF-2. High levels of IGF-1 in the bloodstream have been linked with increased risk of breast cancer, and many laboratory and clinical studies have implicated its receptor in the development, maintenance and progression of multiple cancer types. Signaling by the IGF-1 receptor regulates a variety of cellular processes, including growth, motility and protection from cell suicide. In fact, such signals have been
shown to protect tumor cells from the effects of chemotherapy and radiation therapy. Conversely, inhibiting IGF-1 receptor activity during radiation therapy or chemotherapy has been found to enhance tumor cell suicide rates in animal studies.

In addition to exploring IGF-1 receptor inhibition as a direct therapeutic tool for breast cancer, scientists are evaluating ways to apply it toward preventing or reversing resistance to other treatments, such as endocrine therapies, trastuzumab and lapatinib. Cross talk between the IGF-1 receptor and the various other growth factor receptors—including estrogen, HER2 and additional EGFRs—is a key mechanism for the growth and survival of breast cancer. This codependence and communication between different intracellular pathways is thought to play an important role in drug resistance. Our research group has shown, for example, that blocking the IGF-1 receptor with a monoclonal antibody restores the sensitivity of resistant cells to trastuzumab and disrupts the interaction between the IGF-1 and HER2 receptors. Suppressing the IGF-1 receptor also kills the resistant cells. Furthermore, lapatinib appears to have inhibitory effects on IGF-1 signaling in the trastuzumab-resistant cells, suggesting that its ability to limit tumor cell proliferation may result not only from its anti-EGFR/HER2 activities but also from direct IGF-1 receptor inhibition.

The tangle of signaling pathways leading from the receptors we have been describing to the cellular processes that actually cause a cell to divide or to resist suicide despite DNA damage is highly complex. But scientists are finding that key genes along those pathways are also frequently mutated or dysregulated in tumor cells. Among the best characterized examples is the PI3K gene, whose encoded protein chemically modifies another protein known as AKT, which in turn modifies a complex called the mammalian target of rapamycin (mTOR). This PI3K/AKT/mTOR pathway plays a critical role in the body’s use of glucose for energy and other important physiological processes in normal cells, but it is pathologically overactivated in cancer cells, prolonging their survival. Because the pathway’s effects are ubiquitous in the body, delivering drugs that inhibit it could disrupt healthy cells as well as cancerous ones—a drawback that has so far limited the use of such agents.

Several mTOR inhibitors are nonetheless being tested in clinical trials, both as single agents and combined with other therapies. At the moment, studies using the mTOR-suppressing antibiotic rapamycin, along with an inhibitor of the IGF-1 receptor, suggest that such combinations yield additive antitumor effects as compared with single agents.

Another approach showing great promise is combining direct antitumor agents with compounds that target elements in a tumor’s environment. Cancers secrete a variety of growth factors to attract the endothelial cells that build new blood vessels in a process called angiogenesis. Overproduction of the

### TARGETED THERAPIES

A growing list of drugs designed to inhibit specific tumor proteins are approved to treat breast cancer patients (bold) or are undergoing clinical trials.

<table>
<thead>
<tr>
<th>TARGET</th>
<th>DRUG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen/progesterone receptor proteins</td>
<td>Anastrozole, Letrozole, Exemestane, Fulvestrant</td>
</tr>
<tr>
<td>HER2 receptor protein</td>
<td>Trastuzumab, Pertuzumab</td>
</tr>
<tr>
<td>IGF-1 receptor protein</td>
<td>IMC-A12, CP-751, B71, AMG 479, h7C10, OSI-906</td>
</tr>
<tr>
<td>PI3K/AKT/mTOR cell survival pathway</td>
<td>BG1226, BEZ235A, RAD001, Rapamycin</td>
</tr>
<tr>
<td>VEGF receptor protein (involved in forming tumor blood vessels)</td>
<td>Bevacizumab, Sunitinib, Vatalinib, Pazopanib, AZD2171, AMG706, AMG386, PTC299</td>
</tr>
<tr>
<td>Other targets</td>
<td>Dasatinib (SRC inhibitor), THERAPE, Dendritic cell vaccines, PS3 peptide vaccine, ALT801 (p53 inhibitor), Ad5CMV-p53 (gene therapy), Anti-p53 T cell reinfusion, AZD2281 (PARP protein inhibitor), BSI-201 (PARP inhibitor)</td>
</tr>
</tbody>
</table>

### DRUG TYPE

- **Aromatase inhibitor**
  - Blocks an enzyme involved in estrogen and progesterone synthesis
- **Monoclonal antibody**
  - Impedes activation of cellular receptors
- **Kinase inhibitor**
  - Inhibits signaling by cellular receptors
- **Vaccine**
  - Stimulates production of antibodies specific to tumor proteins; can be composed of cells or peptide molecules
- **Other**
  - Includes direct inhibitors of other molecules or gene therapy to alter cellular protein manufacture
Global Power

Targeted therapies will be most powerful, in principle, when they are used together in combinations tailored to the tumor features driving an individual patient’s cancer. Clinical trials to test specific drug combinations provide critical information about which treatments work most effectively on different tumor profiles and reveal unexpected interactions between drugs. But trials take time, often years, to enroll a sufficient number of participants to generate statistically significant results. That is why multinational research consortia based in Europe and the U.S. are pooling resources to conduct a 50-country trial, the Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization Study (ALTTO), which has just begun recruiting in the U.S.

Some 1,500 testing sites will treat patients with early (stage I or II) breast cancers that overproduce the HER2 protein, giving them chemotherapy and either trastuzumab or lapatinib alone, or one of those drugs followed by the other, or both drugs together. The trial will provide the first side-by-side comparison of these HER2-targeted treatments that work by different mechanisms.

With a goal of including as many as 8,000 women on six continents, ALTTO has the potential to quickly generate results that can then be applied to patients everywhere. Moreover, this global data-sharing model can highlight differences in treatment responses or toxicity among different ethnic groups, a phenomenon observed with differences in treatment responses or toxicity among certain types of chemotherapy because of genetic variations that affect the way the drugs are metabolized by patients’ bodies. Having such information about the newer targeted therapies will help doctors to further personalize treatment, tailoring it to both the tumor and the patient.

—The Editors

most important of these, vascular endothelial growth factor (VEGF), is thought to make tumors more dangerous, and high levels correlate with worse survival rates in human invasive breast cancers. Genentech’s bevacizumab (Avastin) is a monoclonal antibody directed against VEGF that was first approved for use in colon cancer in 2004. In more recent clinical trials among patients with heavily treated metastatic breast cancer, bevacizumab alone had limited activity, but certain patients who received it in combination with capecitabine chemotherapy showed improved responses. In another study, HER2-negative metastatic breast cancer progressed more slowly in patients who received paclitaxel chemotherapy with bevacizumab than it did in patients who received paclitaxel alone. Based on such results, bevacizumab was recently approved for use in breast cancer patients, and other VEGF inhibitors are also in development, such as Pfizer’s sunitinib (Sutent), a tyrosine kinase inhibitor targeted against the VEGF receptor.

At the same time, very basic biology research is continuing to turn up new molecular targets that both reveal more about the underlying mechanisms of cancer and provide potential leads for drug development. Terumi Kohwi-Shigematsu of Lawrence Berkeley National Laboratory and her colleagues announced one such discovery earlier this year. They identified a gene called SATB1 as the “master regulator” of activity for more than 1,000 genes involved in breast cancer metastasis. Kohwi-Shigematsu showed that the influence of the SATB1 protein encoded by the gene is both necessary and sufficient for breast cancer cells to become metastatic, which makes it an appealing therapeutic candidate.

Progress in the molecular targeting of breast cancer and individualized therapy will generally rely on the continuing development of profiling tools to determine whether a patient’s tumor overproduces proteins such as HER2, SATB1 and others that might be direct drug targets. In addition, genetic testing can help characterize a tumor’s overall gene activity patterns—a potential signature of a good or poor prognosis. Still other tests already available or nearing approval can help profile the patient herself to establish whether she has genetic variations that might make her body process a medication more slowly than average—a situation that can be problematic with a drug such as tamoxifen that depends on the body to convert it to active form.

Meanwhile further clinical trials of various drug combinations are needed to validate the effectiveness of multipronged attacks. A 50-country trial has recently begun recruitment in the U.S., for example, to test lapatinib and trastuzumab alone and in combination with each other and with traditional chemotherapies [see box on this page].

Such a large trial exemplifies the considerable resources and attention focused on breast cancer research, in recognition of its importance as a global health threat. Doctors’ ability to profile a tumor and tailor treatment to fight it with a growing arsenal of weapons is already making a difference in the survival rates of patients, and the coming decade promises even more dramatic progress.

MORE TO EXPLORE


