

**SINGLE CANCER CELL
SEEKS MULTIPLE PARTNERS.
AMBITIOUS, HYPER-
ACTIVE, UNSTABLE MUTANT
WITH NOMADIC TENDENCIES.
GOOD AT FITTING IN
(FOR A WHILE). INTERESTED
IN HAVING LOTS
OF OFFSPRING.**

It might be helpful to think of cancer as a ne'er-do-well among us. The one your doctor warned you about.

INSIGHT INTO THE NATURE OF A KILLER

BY JOE MIKSCH AND CHUCK STARESINIC

BAD COMPANY

In 1971, President Richard Nixon declared war on cancer.

This nation has spent billions of dollars to contain the enemy since that day. Cancer is expected to cause about 565,650 deaths in the United States in 2008. On the plus side of the ledger, effective treatments and even cures have been developed for leukemia, prostate and breast cancers, and other forms of the disease. We have learned much. And we have learned that much more escapes our grasp.

Ronald Herberman—director of the University of Pittsburgh Cancer Institute (UPCI), associate vice chancellor for cancer research, Hillman Professor of Oncology, and professor of medicine at the University of Pittsburgh School of Medicine—was at the National Cancer Institute when war was declared.

Herberman says the strategists of the War on Cancer envisioned a series of concentric circles, with the outside ring representing the state of knowledge about cancer at the time. If they could solve the first ring, the second, the third, then they'd be homing in on the enemy and its mysteries—and hit the bull's eye.

No one has hit the bull's eye. It's likely, considering the growing number of known variables associated with the onset and progression of cancer, that there isn't just one to aim for.

TYPOGRAPHY | ELENA GIALAMAS CERRI

Cancer is more complex and cunning than that. Today, scientists have gleaned a much better sense of its nature—which should translate to better care for patients. Rather than thinking of cancer as a foreign enemy on a battlefield, it’s probably more helpful to think of cancer as a ne’er-do-well among us. The one your doctor warned you about.

Cancer is a bit like a predatory character in a thriller. Imagine someone who, lacking any virtue, tries to seduce others to let him hang around. He adds nothing of value and yet will do anything—hide, temporarily change his character, even behave like a bully—for some companionship. But if he succeeds, he’ll end up corrupting everyone around him. The more he gets to know you, the more dangerous he becomes. Not the kind of character you want to get involved with.

Pitt researchers give us insight into this bad seed and how we might take advantage of cancer’s own nature to do away with it.

FORMATIVE INFLUENCES

Before the War on Cancer, there was the Special Virus Cancer Program, which was launched by the NCI (National Cancer Institute) in 1964. The goals of this well-funded, high-profile program were to isolate human cancer viruses and develop a human cancer vaccine or antiviral magic bullet. As it turns out, however, viruses aren’t behind most human cancers.

Why then, decades later, is molecular virology such an integral part of cancer research? At UPCI, it is one of four basic research programs, housing some 28 scientists. For virus hunters Patrick Moore, who leads the program, and Yuan Chang, his wife and close collaborator, it all goes back to the virus they discovered in 1993. (Seven viral precursors to human cancer have been discovered since the 1970s—two by Chang and Moore, both MDs who are Pitt professors of pathology and of microbiology and molecular genetics, respectively.) Moore has been known to say of this virus—KSHV, or Kaposi’s sarcoma herpes virus—that it looks like it was made by a demented tumor biologist, because it contains all the human genes you would want to make a tumor. Over eons of infecting human cells and hijacking our cellular machinery, KSHV has pirated human genes related to cell-cycle control, cell proliferation, programmed cell death, and immune system modulation.

Cancer viruses like KSHV and the polyomavirus responsible for Merkel cell carcinoma, which Chang and Moore revealed on the pages of *Science* in 2008, can point scientists toward the human genes and, therefore, the cellular mechanisms involved in cancer progression.

This way, researchers are learning the biology behind cancer’s deviousness.

TURN-ONS AND TURNOFFS

Sometimes, relationships can go too fast and spiral out of control. Other times, it seems like the parking brake is always engaged, and you're dragging a heavy load. Oncogenes and tumor-suppressor genes are the accelerators and brakes of cancer.

To put the brakes on cancer, you'll want to know about a protein called p53. Its job is twofold. When things are going well genetically, p53 keeps cell growth in check by helping kill cells that are heading out of control; it initiates apoptosis, or programmed cell death. And when cells are functioning normally, the protein works to prevent apoptosis, permitting a cell to live out its normal life cycle.

The gene that produces p53 is a tumor suppressor gene, meaning its regular functioning helps curtail cancer. When the gene that codes for p53 is inactive or malfunctions, cancer results. About half of all cancers are related to disruption of the p53 gene.

Conversely, genes that when turned on become hyperactive and produce proteins that encourage cellular overproliferation are called oncogenes.

So in the case of p53, cancer results when a gene fails to do its job right. With oncogenes, it's a matter of the gene doing its job too well.

"To put it in a user-friendly way, the formation of cancer and the progression of cancer are probably the end result of an imbalance between the regulation and deregulation of the accelerator, which would be the oncogene, and the brakes, which would be the tumor suppressor gene," says Jennifer Rubin Grandis (MD '87, Res '93), a professor of otolaryngology and pharmacology in the School of Medicine.

Grandis' colleague Robert Ferris says that 60 to 70 percent of head and neck cancers are related to a mutation in the p53 pathway. The human papillomavirus (HPV) can cause such mutations. An HPV viral protein called E6 chops up and degrades the p53 gene, rendering it unable to produce the tumor-suppressing protein. Smoking, says the MD/PhD associate professor of otolaryngology and immunology, can also disrupt p53.

Oncogenes, reports Grandis, represent a more complex problem. In most human cancers, she says, there is no discrete mutation of the oncogene. Instead, there's a higher level of activity and an increased production of problematic proteins. The causes of this increased activity are manifold, among them: the swapping of unrelated chromosomes on genes, exposure to carcinogens, and viruses.

Ferris says that understanding the mechanisms that account for these errors is key to developing new treatments.

UNSTABLE MABEL

We've all encountered a few unstable characters. As a general rule, they are bad news, and that is certainly true of cancer. Genetic instability is something all cancers have in common, regardless of whether that instability is attributed to a virus, cigarette smoke, unfortunate inheritance, environmental exposure to carcinogens, ultraviolet light, or inexplicable bad luck. Whether cancer is in the blood, the brain, or the bladder, a population of cells in the body has lost its way and begun to proliferate out of control to the point where it interferes with normal body functions.

There are myriad ways for the genome to become unstable. DNA constantly breaks down. Cell division gone awry can leave a cell with extra copies of genes, including genes responsible for proliferation. Another cell may be incomplete, perhaps lacking genes that limit proliferation.

Living things, through millions of years of evolution, have evolved a whole host of genes associated with maintaining genomic integrity. Scientists are now attempting to identify these genes and understand how they are involved in cancer.

A tumor, by definition, is all of those cells that are genetically unstable in the worst possible ways—able to both escape destruction and proliferate.

SURVIVAL OF THE FOULEST

Being unstable has its advantages. Genetic instability allows a tumor to evolve, just like random mutations have allowed living organisms to evolve through the ages.

Cancer cells experience natural selection. Those that don't proliferate aren't successful. Same goes for those that are so unstable they self-destruct. Some are killed by the immune system or chemotherapy. The survivors are a bad bunch—they are able to evade the immune system, survive chemotherapy, and rapidly proliferate.

"The major limitation in the approach of immunology, but also in the approach of chemotherapy, is the ability of tumor cells to develop escape mechanisms because of genetic instability," says Soldano Ferrone, an MD/PhD Pitt professor of immunology who is an expert in molecules manufactured to turn the immune system against cancer. He and other Pitt immunologists are working on a two-pronged immunotherapy for melanoma as well as breast, head, and neck cancers. By combining antibodies with stimulation of the immune system's killer T cells, they believe they can counteract any escape mechanisms the cancer may evolve.

THE BAD SEED

Remember when you were a kid? Your parents may have told you that you could be anything when you grew up: president, CEO, firefighter, Pittsburgh Steeler.

Well, back before you were even a kid—when you were a teeny blastocyst—you were a mass of embryonic stem cells, which truly could become anything: lung tissue, heart tissue, bone, brain, or other. Even as a grown-up, other cells known as adult stem cells allow your body to replenish blood, skin, hair, muscle, gut ...

Nice tricks. But not so nice when it comes to cancer. Pitt researcher Eric Lagasse seeks to understand the evil stem cells scientists believe lead to cancer. These are genetically unstable mutants that may be responsible for the spread and recurrence of cancer.

Lagasse, a PhD professor of pathology, notes that chemotherapy is often successful in shrinking tumors. Yet, cancer frequently returns. Chemotherapeutic agents target actively proliferating cells, but cancer stem cells evade the chemical onslaught. They only divide and proliferate when necessary and, therefore, seem immune to the usual run of cancer therapies.

Let's say a patient is treated for colon cancer. Surgery and chemo render that person free of any detectable cancer. Lagasse likens what happens next to when a person suffering from a bacterial infection quits her course of antibiotics as soon as she's feeling better.

"The problem (with cancer) is the short exposure to chemotherapy," Lagasse says. He explains that if you go any longer than three weeks, normal dividing cells and, eventually, the patient die. "But after several rounds of chemotherapy, you have a cancer that is incurable. You're left with a very strong, selected population of cancer stem cells that are very resistant and will kill the patient."

Vera Donnenberg, PhD assistant professor of surgery, says one of the major problems associated with killing cancer stem cells is they have taken on the guise of normal adult stem cells and can protect themselves by the same mechanisms healthy cells use. So, if you kill cancer stem cells, you kill normal cells.

"Vital organ functions cannot be disrupted for long without lethal consequences," she says. "It is not the death of normal tissue stem cells that kills the patient. It is the race between normal tissue repair and killing or injuring the tumor stem cells."

And these dodgy cancer stem cells live on to create new tumors and go to new places, colonizing other tissue.

Lagasse says much work must be done before a drug can be developed to target these cancer seeds while sparing adult stem cells and normal tissue.

"Drugs come at them. But because of their genetic instability, they can mutate so fast that they can survive," he says.

CON MAN

Cells that form tissue are kind of like the three musketeers. They're one for all and all for one as they stick together to be skin, or a heart, or a liver. Among the glues that hold cells in formation is a surface molecule called E-cadherin.

It makes sense to scientists that spreading cancer cells turn off the production of E-cadherin; otherwise, the cells wouldn't be able to grow unchecked or make their way out of their orderly home in healthy tissue.

Pitt's Alan Wells (an MD/DMSc, the Thomas J. Gill III Professor of Pathology, and vice chair of the Department of Pathology) and his former student Christopher Shepard (PhD '07) recently discovered that cancer cells don't cancel E-cadherin production permanently, as had been thought. After a cancer cell travels from its original home, it can insinuate itself into healthy tissue, where it resumes E-cadherin production. This allows the cancer cell to appear to be normal when it joins healthy tissue.

Imagine a cancer cell migrating from a prostate tumor to the liver. Once it resumes E-cadherin production and blends in with its neighbors, it relies upon survival signals from surrounding liver cells and is able to evade chemotherapy, which does not kill normal, nondividing cells.

Wells' lab is continuing its investigation into the signaling process that permits the resumption of E-cadherin production. He also hopes to discover the catalyst that causes cancer cells that have weaseled their way into healthy tissue to begin to reproduce unchecked and create a new tumor.

Therapeutically, says Shepard, now a visiting scientist at the Massachusetts Institute of Technology, this cellular trickery presents a problem. Because E-cadherin is vital to normal cellular function, slowing down or stopping its production could disrupt all manner of physiological processes in normal cells.

"The targeting mechanism would have to be very intricate," Shepard says.

CAN IT EVER BE FIXED UP?

When it comes to cancer, the answer is ... maybe.

DNA damage occurs constantly, whether from ultraviolet light or the byproducts of metabolizing your lunch. It doesn't usually lead to cancer, because your beleaguered cells are capable of doing all kinds of things when the genome goes bad. Hundreds of genes are devoted to DNA damage repair. Each is responsible for a different aspect of repairing the long, twisting ladders of microscopic DNA inside each cell. Some proteins "climb" the ladder and flag the errors. Some nucleases excise faulty base pairs, and others break the inter-strand crosslinks that kink the ladder.

Laura Niedernhofer, an MD/PhD and Pitt assistant professor of microbiology and molecular genetics, theorizes that some deficiencies in DNA repair can lead to cancer. In her lab, mice that are disabled in DNA repair succumb to rapid aging and death, but not cancer, if they suffer damage to both strands of the DNA ladder.

Mice with damage to a single strand live longer but get cancer.

"The cells won't die," she says, "but you're at a huge risk, then, of mutations being fixed into the genome, and that's what contributes to genomic instability and cancer."

In the lab, basic scientists have developed drugs to inhibit certain aspects of the DNA repair process. This allows them to tease apart the pathways and better understand what's happening, but these approaches are not yet used clinically. The NCI is encouraging investigators to get to the next step. This fall, Niedernhofer will take part in a special NCI workshop devoted to DNA repair as an "emerging translational field."

Theoretically, a doctor might increase DNA repair to protect cells from becoming genetically unstable, or she might down-regulate DNA repair in tumor cells so they are unable to survive the onslaught of chemotherapy. In other words, a tumor might get fixed up or fixed down.

A LITTLE CHEMISTRY

After biopsy, some tumors reveal a molecular signature that tells clinicians which chemotherapy treatments are most likely to work.

Like insects that can read the molecular signatures of one another in the dark to determine so much—species, gender, health, availability for mating, status in the colony—scientists are rapidly learning to identify the endless organic molecules in existence, including those that are associated with specific cancers and stages of cancer.

A slew of researchers at UPCI are finding ways to use such biomarkers for early detection. For example, Anna Lokshin, a Pitt assistant professor of medicine in the hematology/oncology division, has developed a simple blood test that can reliably diagnose early-stage ovarian cancer in more than 97 percent of women with cancer who undergo it.

ENABLERS

A tumor doesn't exist in a vacuum. It needs help.

"The tumor is not there by itself," says Theresa Whiteside, a PhD professor of pathology, otolaryngology, and immunology at Pitt. Tumors have hosts—namely our bodies—and those hosts can be gracious to a fault.

It was once assumed that immune cells found on the tumor were there to fight the tumor. But evidence shows that some immune cells are actually recruited by the tumor and help it grow. Other cells in the immediate environment of the tumor are clearly not cancerous, but they help create new blood vessels to feed the tumor.

"We have already learned a great deal about the tumor micro-environment and how to explore it to benefit the host, not the tumor," says Whiteside.

With Pitt's Soldano Ferrone, she has developed a vaccine able to target a unique molecule that appears on head and neck tumor cells as well as on the normal cells building blood vessels to sustain it.

MARKED AND ESCORTED OUT

In the old days, the only vaccines around were those that turned the immune system against foreign invaders such as smallpox or polio viruses. A vaccine essentially showed the immune system a sample of the virus in killed or incapacitated form so that it would recognize the real deal if it ever showed up.

But cancer isn't a foreign invader. It's your own cells gone bad. To teach the immune system to target cancer, scientists must first identify molecules that distinguish cancer cells from normal cells. Even then, the markers might appear in some form on normal cells. The first clinical trial in the world of a synthetic peptide cancer vaccine—at Pitt in 1993—enrolled patients with very advanced cancer and poor prognoses. Regulators deemed the risk of a vaccine precipitating an immune attack against healthy tissue too great to include patients with better odds.

“The first patient was a stage-four breast cancer patient who had two pumps: one emptying liquid from her lungs and one emptying liquid from her abdomen,” says Olivera Finn, professor and chair of the Department of Immunology at Pitt. “She had chest disease like you wouldn't believe. She was an absolutely end-stage cancer patient, and she died within a month of us starting the trial.”

A lot has happened since then, and cancer immunotherapy is now more realistically expected to prevent cancer, prevent recurrence, or cure it in its early stages. For example, Pitt's John Kirkwood, professor and vice chair for clinical research in the Department of Medicine, developed the first adjuvant therapy for patients recovering from melanoma, a disease likely to recur. The interferon therapy stimulates the immune system's natural killer cells (see below) against melanoma. And Finn will initiate a vaccine trial this year against colon cancer.

**DON'T YOU
MESS AROUND
WITH MY
LITTLE SISTER**

They prowl your body like paranoid and protective big brothers. And, by design, they don't like intruders. Dubbed natural killer (NK) cells, they were discovered in Ronald Herberman's laboratory at the National Cancer Institute in the early 1970s, before he established UPCI. For cancer researchers, NK cells were an exciting revelation.

To fight a virus, most immune cells need to ramp up the system first. But a tiny percentage of immune cells—the natural killers—are rapid responders, born ready to identify and destroy aberrant cells, including cancer cells.

Researchers are slowly learning about the complex chemical signals that activate NK cells. A successful treatment for bladder cancer, for example, has been found to owe some of its success to NK cells. Stem cells have been coaxed into becoming NK cells in the lab, and such customized cells may be introduced into cancer patients one day. ■