Researcher's quest to understand cancer by unraveling the mysteries of cell division

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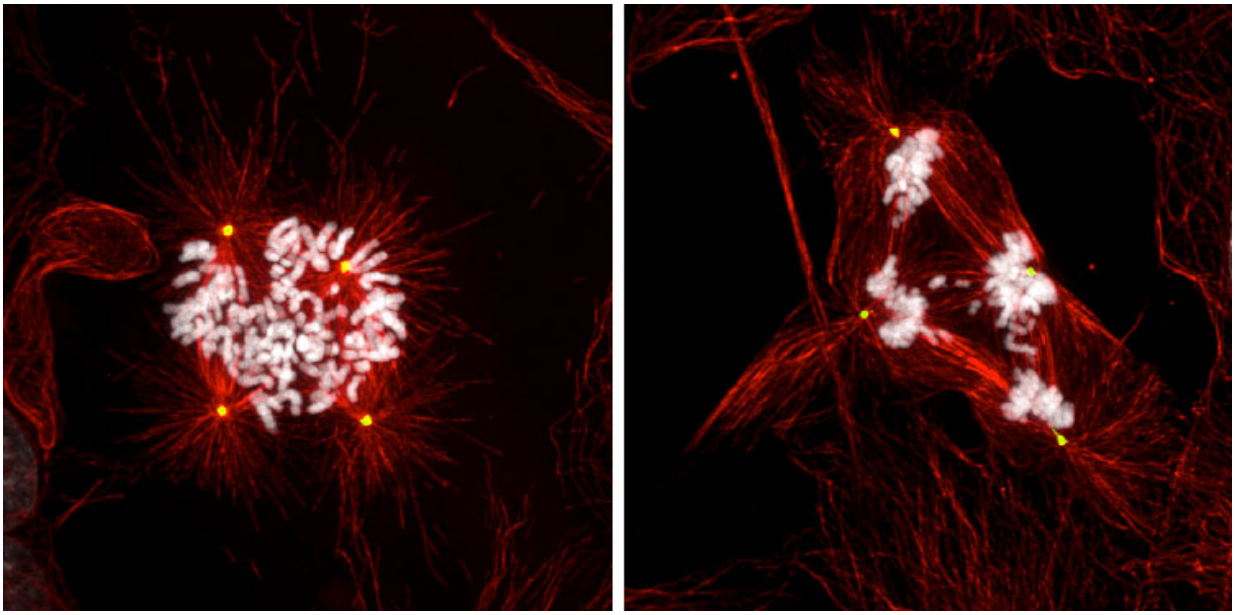

Left: When cells divide incorrectly, it can lead to cell death or cancer. During cell division, centrosomes—seen here as yellow-green dots—attach to chromosomes—the white blobs—and pull them apart. Normal cells have two centrosomes, cancer cells often have four. Right: Scientists assumed that the extra centrosomes led to four abnormal daughter cells, which then gave rise to tumors. Ganem found this wasn't true; the four daughter cells all self-destructed before becoming cancerous. Instead, he discovered another mechanism that led to cancer, turning the conventional wisdom on its head.

There comes a defining moment for many scientists that divides their

lives into before, and after. Neil Ganem remembers that moment. He was a PhD candidate at Dartmouth Medical School, with vague ideas of studying "some sort of neuroscience." He thought he might pursue Parkinson's disease, which had killed his father. But then came Duane Compton's black and white movie.

Compton, a professor of biochemistry at Dartmouth and interim dean of the college's medical school, has studied cell division for more than 20 years. In particular, he studies something called chromosome segregation—how cells separate their DNA into two equal heaps before dividing into two daughter cells. "We want to know how this works so well in normal cells, how they segregate so perfectly every time they divide," Compton says. Each fall, he presents his research to aspiring Dartmouth PhDs, starting with a simple movie of cell division. "It was just one cell," recalls Ganem. "You could see the nucleus and then you could see all the chromosomes. You could see them all move around, line up perfectly, and then suddenly that one cell pinched into two."

The gritty, grainy movie mesmerized Ganem. Then Compton spoke. "Why do we study this?" he asked the assembled students. "Cancer. Cancer is just a disease of cell division gone wrong."

"And that's all he said," recalls Ganem. "And that's all I needed. I was hooked."

Since that day at Dartmouth in 2000, Ganem, a Boston University School of Medicine (MED) assistant professor of pharmacology and experimental therapeutics, has tackled cancer in his own unique way: by capturing stunning images of cell division, examining them with a critical eye, and asking questions that nobody thought to ask before. His work has upended our understanding of how cells become cancerous, earning the 37-year-old scientist influential articles in Cell and Nature, as well as a cascade of grants, awards, and accolades. These include the

Smith Family Foundation Award for Excellence in Biomedical Research, the Melanoma Research Alliance's Jackie King Young Investigator Award, and the prestigious Searle Scholar Award, given to the country's most promising young chemistry and biology researchers.

"He has the ability to identify big, broad questions, express them in a simple way, then proceed logically with testing his hypotheses. That's a rare talent," says David Farb, a MED professor and chair of pharmacology and experimental therapeutics. "His research opens up new avenues for treating cancer, and he does it in a beautiful and rigorous way."

Cell division, when all goes well, is breathtakingly beautiful. It starts when the DNA in the cell's nucleus, usually a scramble of stretched-out spaghetti, duplicates itself, then coils into tightly packed structures shaped more like stubby macaroni. Matching pairs of macaroni join at the middle with a little nub of protein, forming the familiar X-shaped chromosomes.

Meanwhile, two tiny structures called centrosomes migrate to the poles of the cell. Then the amazing part happens: the centrosomes at either pole grow tiny tubes called microtubules that reach toward the center of the cell, building long spidery spindles that attach to the center of each chromosome. It's sort of like spearfishing, with the centrosomes as fishermen casting multiple lines that hook the waiting chromosomes. When all the chromosomes are hooked, they line up in the middle of the cell. Then—one, two, three, presto!—the centrosomes pull them apart, the cell pinches down the middle, and you now have two identical daughter cells.

The whole process, start to finish, takes about 20 minutes, and is so complicated and choreographed that most people—even seasoned cell biologists—are amazed it all works. But it does work, most of the time,

because the cell has built-in checkpoints along the way. If the cell senses something amiss—like too many chromosomes in a cell or not enough—it self-destructs. Cancer happens when the self-destruct mechanisms stop working and mutant cells that should die do not. Instead, they keep dividing, out of control. Most anti-cancer drugs target this characteristic, attacking all the rapidly dividing cells in the body. This kills cancer cells but also destroys healthy hair follicles, skin cells, and the lining of the mouth and gut, leading to painful and dangerous side effects.

"The cancer problem is so hard to crack, because it's not like a foreign bacteria invading our cells. It's a deregulation of the cell's normal machinery," says Compton. "It's not easy to figure out how to stop cancer without stopping everything else. It's hard to find its Achilles' heel."

Almost all tumors have an incorrect number of chromosomes, a condition called aneuploidy—"that's one of the hallmarks of cancer," says Ganem. Understanding aneuploidy is a leading area for cancer research; the oddly numbered chromosomes make cancer cells stand out, offering possible ways to attack them selectively. The idea appealed to Ganem, who joined Compton's lab and began to study the nitty-gritty details of how chromosomes move into daughter cells. He focused on proteins called kinesins, which help build and dismantle the spindles. Sometimes kinesins go wild, building abnormal spindles, which can connect to chromosomes incorrectly and pull too many into a daughter cell. Ganem studied the process through high-resolution imaging, taking pictures of spindles attaching to individual chromosomes.

Ganem calls himself a "visual person," who prefers books to podcasts and microscopy to mental math. His mother, a grade school science teacher, bought him a microscope when he was a young boy, "one of the best gifts I ever got," he remembers happily. "It came with a bunch of

cover slips and empty slides, so I spent a ton of time out in the backyard just finding stuff, putting it on there and looking at it." Squashed bugs, spit, money—Ganem grabbed everything in reach and studied it under the scope, marveling at the fine details of everyday objects, like the tiny creatures swimming in a drop of pond water. "It's just amazing what's there to be seen," he says. And he never stopped looking.

"When I put a cell on a microscope and watch it move with my own two eyes, I understand it better," says Ganem. "Now that I can see how it's moving, jeez, now I have an idea about why it's moving incorrectly. As you watch it, the ideas just come—at least come to me—a lot easier."

In Compton's lab he met his future wife, Amity Manning, now an assistant professor of biology and biotechnology at Worcester Polytechnic Institute. "The imaging was a big draw for both of us. You can interpret numbers, but when you can also see the results, it has that much more power," she says. "Plus you're looking at things nobody has seen before. It's like being a space explorer but the other way around."

The live-cell images Ganem produced at Dartmouth allowed him to explain how two novel kinesins help assemble spindles and move chromosomes, and he made several important discoveries detailing how deregulation of these kinesins contributes to aneuploidy and cancer. Ganem carried this background with him to Harvard in 2006, for his postdoctoral fellowship under David Pellman, a professor of pediatric oncology and cell biology at Harvard Medical School and the Dana-Farber Cancer Institute. "Neil had been studying the basic mechanics of cell division and was starting to think more about cancer," recalls Pellman, who appreciated the "sparkle of intellect" he saw in the young scientist. "He came to my lab to make the connection between basic cell biology and cancer biology."

Most cancer cells, in addition to their myriad other problems, have extra

centrosomes—those spearfishermen at the poles that cast out the microtubules and pull chromosomes apart. Most scientists assumed that the extra centrosomes formed three or four poles in a dividing cell, leading to three or four abnormal daughter cells, instead of two healthy ones. Those abnormal daughter cells then gave rise to tumors. Or so everyone thought. The idea seemed plausible, but Pellman and Ganem weren't so sure it was true.

"We wanted to pick apart what was going on," says Pellman. "We thought that centrosomes played a role, but every cancer cell—like every unhappy family from Tolstoy—has its own unique story. Lots of strange things go on in a cancer cell, and it's hard to tease apart the centrosomes' role."

So Ganem got on the microscope and got to work, watching thousands of cancer cells with extra poles divide, and then tracking their daughter cells to see if they survived. Nobody had ever done this before, partly because the technology hadn't existed. The work relied on a new microscope with a cell incubator attached, which allowed Ganem to follow the fates of dividing cells over several days. It also required grit. "This was really tedious, boring work," says Ganem. "It gave me motion sickness." At night, visions of dividing cells swam through his mind's eye, keeping him awake.

Ganem's work led to a discovery that turned the conventional wisdom on its head. He found that the multipolar divisions did sometimes lead to three or four daughter cells with abnormal numbers of chromosomes. But those mutant daughter cells always died, never becoming tumors as everyone expected. "So basically that idea was just wrong," says Ganem.

Peering at the cells, Ganem saw something else instead. Many of the cancer cells had four centrosomes, appearing as if they would divide into four abnormal daughter cells. But the ones that became cancer didn't do

this. Instead, they clustered the extra centrosomes at two poles and divided into two daughters. Scientists had seen this before, but Ganem discovered that cancer cells did this most of the time. He discovered something else, as well: exactly what those clustered centrosomes were doing, and how it led to cancer. Because the extra centrosomes sent out extra microtubules, they hooked chromosomes every which way and reeled them in willy-nilly—a process called merotelic attachment. The daughters survived, but their rate of chromosome missegregation skyrocketed. This mechanism is now widely accepted as the major underlying cause of chromosome missegregation in human cancer cells.

"Neil made the connection between the centrosomes and merotelic attachment," says Pellman. "He had the insight. He realized the significance and made it work." Ganem published the results in a 2009 cover story in Nature. The article is the most cited paper on centrosomes in the last ten years—an indication of its significance in the field—and the most cited paper to ever come out of Pellman's lab. "I'll probably be doing science for the next 40 years, and I'll likely never make a discovery as important as this one," says Ganem. Pellman disagrees.

"I know Neil very well. He's creative, he has interesting ideas and insight. He's a rigorous scientist and his own strongest critic," says Pellman. "He's the guy who's going to get the right answer."

Ganem looks young for his age, with a round boyish face and a wide smile. He's enthusiastic and cheerful, with an "aw, shucks" demeanor and office décor based mostly on photos of his three young sons, and their artwork, like the brightly painted rocks propping open his door. So it's surprising to hear his colleagues and mentors list his defining qualities as intensity, intellectual ferocity, and scientific rigor.

"He's naturally very optimistic, but when it comes to science there's something intrinsically skeptical about him," says Manning. "That's what

makes him check all the data and make sure all the controls are in place before moving forward."

Ganem and Manning juggle dual science careers in addition to raising their children. Ganem spends much of his free time trying to recreate the best parts of his New Hampshire boyhood for his kids—tromping around the woods, reading Dr. Seuss, and playing basketball. Manning says her husband also builds lots of LEGO cars and trucks with the boys, an especially appealing pastime for Ganem. "It's very visual," says Manning. "You have these little building blocks and you put one section together, then you put the bigger sections together, and then you get to see the final product. It's like that in the lab. You build your understanding little by little until you see the final picture."

That is one of Ganem's greatest gifts as a scientist, says Dartmouth's Compton: puzzling together disparate pieces of information into a coherent whole. "When I saw what Neil did with that 2009 Nature paper, I said 'aha!'" recalls Compton. "It was his insight that put together all the different pieces and related them in a way nobody else had done before."

At BU, Ganem continues to build on the work he began at Dartmouth and Harvard, now focusing on tetraploid cancer cells—those with four sets of chromosomes instead of two. When tetraploid cells divide, they can lead to cancer. "If you look at any solid tumor—doesn't matter if it's from the brain, from the lung, from the breast, from the pancreas—and you count the number of chromosomes in each cell, the numbers will vary, depending on the cancer," says Ganem. "But at least half, if not more, will have a near tetraploid number."

Usually when a tetraploid cell forms, it never divides again. Ganem wondered why. "Some tumor suppression mechanism kicks in and just shuts down the whole thing," he says. "I was really curious about this. I wanted to know: What is stopping tetraploid cells from proliferating?"

After several years of examining, purifying, and screening tetraploid cells, Ganem found an answer: the Hippo pathway, a cascade of cell signals that controls the size of organs in animals. First discovered in fruit flies, the name comes from a gene called Hippo—yes, as in hippopotamus. When mutated, it causes the unfortunate flies to grow monstrous eyes or wings.

Ganem and his colleagues discovered that the Hippo pathway regulates not only organ size, but also the growth of individual cells. Most tetraploid cells, because they are simply too big, turn on the Hippo pathway and self-destruct. Cancer cells, Ganem found, turn the pathway off, and keep growing and dividing despite their already enormous size.

Ganem hopes that this line of research, published in 2014 in the journal Cell, may point the way to new cancer therapies that target abnormal tetraploid cells while leaving healthy cells alone. This remains the holy grail of cancer therapy. Though President Richard Nixon memorably declared a war on cancer in 1971, the disease has proven an intractable enemy, killing over 1,500 people in the United States every day. Ganem's research may someday put a dent in that staggering statistic.

"Our long-term goal is to identify new ways to specifically kill cells with an abnormal number of chromosomes, while sparing the normal cells from which they originated," he says. "To do that, we first need to identify what makes cells with too many or too few chromosomes unique. And taking a good, hard look at them under the microscope is a good place to start."

More information: Neil J. Ganem et al. A mechanism linking extra centrosomes to chromosomal instability, *Nature* (2009). DOI: 10.1038/nature08136

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