How Cancer Doesn’t Happen – Part 2

Our bodies can keep tumors at bay, even if our cells harbor cancer-driving mutations.

September 6, 2022 By Fred Hutch News Service

We’re all mutants. Each of us is brimming with genetic hiccups slowly accrued over our lifetimes. Many of these blips in our DNA are strongly linked to cancer — so why are we walking mutants but not walking tumors?

It’s not easy to study cancer that doesn’t happen, but some scientists, including a few at Fred Hutchinson Cancer Center, are examining how our bodies keep mutated (and even cancerous) cells from succumbing to their darker instincts. Their insights into how our bodies manage mutant cells could point the way toward better strategies to prevent cancer or keep it in check. We talked to several of these scientists to learn more.

Microenvironment: Cellular Peer Pressure

A properly functioning tissue is a group effort.

Mutated cells don’t live or die, grow or age by themselves. They get a little help — or a little hindrance — from their neighbors.

“The idea that you need a permissive environment [to promote cancer] has been around for a long time,” said Fred Hutch’s Dr. Cyrus Ghajar, who studies tumor-cell dormancy.

His postdoctoral mentor, Dr. Mina Bissell, showed this in the 1980s while studying a virus that causes tumors in birds. When scientists injected chickens with the virus, a tumor would grow wherever they stuck the needle. But the virus traveled throughout the birds’ bodies, Bisselll found — so why did tumors only grow in one place? She found that if she scratched a bird on one wing, and injected the tumor virus on the other, two tumors would grow: one at the injection site and one at the scratch.

Bissell’s work showed how important the cells and biological factors surrounding a tumor — its microenvironment — are to its growth and survival. But her findings also highlight how inhospitable normal tissue is to tumor development. It would be easy to assume that tumor suppression is a passive process, that mutated cells are dry kindling waiting for a spark to ignite a tumor.

But it turns out our tissues are continually snuffing out sparks before they blaze into full-blown tumors.

Fred Hutch skin cancer researcher Dr. Slobodan Beronja discovered this while trying to study the forces within skin that could promote cancer development. He focused on skin stem cells, which are the source of the cells that make up our skin tissue. In addition to producing more skin cells, stem cells can also renew and multiply themselves to maintain a pool of cells that can continue producing skin tissue.

A stem cell that picks up a mutation doubles that mutation — and the number of mutated stem cells — every time it doubles itself. If the mutation can promote cancer, this would double the number of stem cells at risk of turning into tumors. Beronja theorized that a biological mechanism that increases the number of stem cells in a tissue could also increase the risk of skin cancer.

“I was looking at stem cell renewal as a completely passive mechanism that would facilitate more efficient tumorigenesis [cancer development],” he said.

He had developed a technique that allowed him to measure the rate at which new stem cells form. He introduced into the stem cells cancer-associated mutations in a gene called Ras, which is known to amp up cell growth and promote cancer when overactive.

“To our surprise, there were fewer stem cells as a result [of mutating Ras]. And the rate at which they were replenished went down,” he said. “At first, of course, I thought something went wrong with the assay, because this makes no sense.

Around the same time, another group discovered that a single square inch of sun-exposed, non-cancerous skin from the eyelid of a middle-aged adult can abound with hundreds of strongly cancer-associated mutations. Most people receive plenty of UV rays, but few develop tumors of the eyelid. The findings suggested to Beronja that skin tissue has evolved ways of handling mutations, which are inevitable.

So he flipped his approach. Rather than focusing on how mutations cause cancer, Beronja instead asked how skin prevents mutated cells from taking over.

He’s already discovered that several different strategies skin uses to handle mutated cells, and expects to uncover more. One involves an interplay between mutant and normal skin cells. Beronja and his collaborator, Dr. Valentina Greco at Yale University, saw that skin appears to use a type of cellular peer pressure to get rid of mutated skin stem cells. They saw that mutated cells within skin structures — such as hair follicles or oil glands — wax, then wane and ultimately disappear. Before a mutated structure ebbed away, a cadre of normal cells surrounded it. These normal cells seemed to gang up to expel the mutant cell and then reestablish normal structure.

The findings highlighted how hard our tissues work to maintain the 3D arrangement of specialized, or differentiated, cells that ensures the tissue performs its proper role in the body — and how this drive to maintain order could be keeping cancer at bay.

“Our organs are set up for function, and that function is inextricably linked to architecture,” Ghajar said. “A lot of that function has to do with a differentiated state. And for many cells, being differentiated is typically decoupled from growth.”

Differentiation occurs as cells progress from stem cell to specialized cell, usually losing the ability to grow and divide along the way. Tissues are set up to maintain cells in a quiet, differentiated state, so when a cancer cell finds its way in, “it falls under that spell,” Ghajar said.

He studies how migrant breast cancer cells often turn dormant when they spread, or disseminate, to a new tissue. His work on dormant breast cancer cells in the brain, bone marrow and skeletal muscle highlights the similarities and differences in each organ’s hypnotizing magic.

In the bone marrow, tumor cells slumber nestled into the wall of the blood vessels they rode in on. This area helps coax cancer cells to hibernate in every tissue Ghajar’s team studied — but each adds a unique ingredient to its spell. In the brain, napping cancer cells nuzzle the toes of astrocytes, star-shaped brain cells that help regulate everything from cerebral blood flow to healing after brain injury. Dr. Jinxiang “David” Dai, a postdoctoral fellow in Ghajar’s lab, pinpointed a protein released by astrocytes that promotes cancer cell dormancy. The finding is a step toward a potential therapy to keep dormant cells permanently snoozing.

And though the brain and bone marrow can put a damper on breast cancer metastases, too often it’s only temporary. And metastatic breast cancer, also known as stage 4, has no cure. But metastasis isn’t an equal-opportunity phenomenon.

“There are tissues that are clearly just not permissive, they’re just a wasteland,” Ghajar said.

One of those tissues is skeletal muscle. Metastases almost never arise here, and when the amount of muscle tissue is factored in (it’s about 40-50% of the average person’s body mass), the rarity becomes even more pronounced. Tumors also almost never originate in skeletal muscle, either.

But it’s not clear why, Ghajar said. Muscles have lots of blood vessels to act as tumor-cell delivery systems. Muscle tissue is constantly being broken down and healed, rebuilt by reawakened stem cells — and scientists have known since Bissell’s studies that a wound is a cancer-cell cornucopia. Perhaps tumors don’t grow here because cancer cells avoid the area?

But according to preclinical work by Dr. Sarah Crist, then a graduate student in Ghajar’s lab and now a postdoctoral fellow at the University of Minnesota, breast cancer cells do make it to skeletal muscle and they can survive — they’re just hanging on by the skin of their teeth.

“If it’s not about your ability to traffic there, and it turns out it’s not about your ability to survive there, it’s really all about your ability to colonize that tissue,” Ghajar said.

Muscle is unlike our other tissues: Muscle cells use a lot of energy and the contractions they perform expose the individual cells to physical stress most other cells don’t experience. After ruling out other possible reasons muscle is inhospitable to cancer cells, Crist and Ghajar found that

the cancer cells' stumbling block was stress: oxidative stress. To power themselves, muscle cells consume lots of oxygen — and spit it out in the form of damaging molecules called oxidants. Muscle cells buffer this damage with higher-than-average levels of protective antioxidants, but cancer cells are only equipped to handle lower level of oxidative stress found in other tissues. Skeletal muscle is a harsh environment most tumor cells don't have the tools to overcome. They have only one option: hunker down and endure.

Crist found that if she gave cancer cells "emergency provisions" by boosting the level of an antioxidant enzyme called catalase, they could grow new tumors in skeletal muscle. But when the team routed catalase-amped tumor cells to the lung, the extra catalase inhibited their growth and reduced lung metastases. Turning their antioxidant capacity up even further killed the lung mets off.

"So what does that tell us? It tells us there's an optimal level of oxidation and reduction [antioxidation] that a tumor cell needs and it's tuned to the tissue it's in," Ghajar said.

He and Crist hadn't just revealed a tumor-suppressing process in skeletal muscle, they had revealed an inner truth about tumor cells and how they relate to their surroundings. Ghajar and his team are now working to figure out how they can exploit this vulnerability to permanently press snooze on dormant tumor cells (or even kill them off) and prevent stage 4 disease.

While there are several therapeutic avenues his team is exploring, Ghajar favors an approach that could work in every tissue that harbors dormant tumor cells. His team turned to the immune system.

The Immune System: A Mobile Microenvironment

Our immune system is an incredibly complex interconnected set of cells and molecules that collaborate on one goal: keep disease at bay. Disease mostly means infection, but the immune system can also defend against cancer.

Because a pathogen or tumor could strike anywhere in the body, immune cells are highly mobile. They ride along blood vessels and squeeze into tissues to heed the call of injured cells or to hunt for hidden dangers. An arriving company of immune cells can change a tissue's microenvironment.

Mice with defective immune systems develop tumors at earlier ages than mice with normal immune systems. The tumors that grow in spite of an immune system are often less able to trigger immune activity, a hint that they succeeded by evolving ways to fly under the immune system's radar. And when researchers look at the cellular components of tumors, the presence of certain immune cells can correlate with a better prognosis, suggesting that these cells are working to restrain the tumor. (Unfortunately, some cancers find ways to convince immune cells to collaborate with them and enhance tumor growth and spread.)

But immune cells are unlikely to act when there's just one mutated or cancerous cell, said Dr.

Shivani Srivastava, a Hutch researcher who studies how to improve cancer immunotherapies. Immune cells like T cells are most effective when they're flagged down and directed where to go; a single cell, cancerous or not, is unlikely to send out the necessary SOS. So, while our immune system can help us once we have a tumor already, it's probably doesn't play much of a role in keeping us from developing them in the first place.

Srivastava studies immunotherapies based on a type of immune cell known as a T cell. T cells carry a specialized molecule on their surface, called a T-cell receptor, that helps them seek out and kill off diseased cells. Immunotherapies in which T cells have been genetically engineered to find cancer cells have been approved for certain blood cancers, but scientists are hoping to expand their scope to other cancer types.

Ghajar hoped that the immune system could hold similar hope for patients with metastasized breast cancer. Dr. Erica Goddard, a postdoctoral fellow in Ghajar's lab, teamed up with Srivastava and Hutch immunotherapy expert Dr. Stan Riddell to study the problem. A single dormant breast tumor cell seems like it would be no match for a deadly T cell. It doesn't have to be, it turns out.

"There's a very simple explanation for how [dormant tumor cells] evade immunity, which is that they're rare," Ghajar said.

It's a numbers game: We have more than 30 billion cells in our bodies that a handful of cancer-attuned T cells must hunt through.

"It's like trying to win the dormancy lottery again and again," Ghajar said. "So how do you tilt things in your favor?"

With numbers. Goddard and Ghajar, with Riddell's team, showed in mouse studies that a host of engineered T cells change the odds from slim-to-none to pretty fantastic.

There's still work to do: T cells need a specific target to home in on. Ghajar has worked with Hutch epidemiologist Dr. Chris Li and an expanding team to recruit women with early-stage breast cancer — who likely have cancer cells slumbering in their bone marrow — to donate bone marrow samples. The scientists can screen these samples for dormant tumor cells and characteristic mutations that T cells could target. The team hopes this work reveals targets they can use to develop an immunotherapy that prevents metastatic breast cancer.

The project is part of TRANCE, a multi-disciplinary, multi-center collaboration supported by a $25 million Department of Defense grant co-led by Ghajar and Li. The TRANCE consortium aims to develop strategies to kill or silence dormant tumor cells.

Context Matters, but So Does the Mutation

When Beronja started studying how cells stopped cancer-causing mutations from actually causing cancer, he expected that to see mutated cells choosing a couple of obvious strategies. Self-

sacrifice is one obvious way a mutated cell could defend its tissue against cancer. Cells can also take what amounts to a permanent time out, a phenomenon known as senescence.

But mutated skin stem cells chose a different option: they persisted, and persistently continued fulfilling their functions as skin cells. The strategy underscores how imperative it is that skin stem cells continue fulfilling their role as the source of skin tissue, Beronja said.

To continually maintain skin's upper layers, skin stem must continually produce differentiated skin cells with specialized functions. But they must also renew themselves to maintain the stem cells that are the source of skin tissue. Skin stem cells have to strike just the right balance between renewing themselves and differentiating. Tip too far either way, and skin will eventually break down. Too much renewal, and skin tissue isn't replaced. Too much differentiation drains the well of skin stem cells — and eventually, skin tissue won't be replaced.

So generally skin stem cells divide asymmetrically: They produce one stem cell and one more-differentiated cell. This adds up to a renewal rate of 0.5.

But mutations can alter a stem cell's renewal rate. Mutated and normal skin stem cells adjust to this change by toggling the balance between renewal and differentiation so that skin continues to keep the outside out and the inside in. But how the balance shifts depends on the mutation.

Skin stem cells with the activated form of Pik3ca, one of the most commonly mutated cancer-drivers, don't grow more. Instead, they differentiate, gaining specialized functions and losing the ability to divide. (Beronja and his then-postdoctoral fellow Dr. Zhe Ying, who led the project, dubbed this "oncogenic differentiation.") This dries up the well of mutated skin stem cells that could seed a tumor. To prevent tissue breakdown, the unmutated cells next to a Pik3ca mutant respond: They tip toward renewal to replenish the stem cells and keep the tissue in balance.

Beronja found a different result with a different oncogene. When Dr. Madeline Sandoval, then a graduate student in Beronja's lab, activated Ras, another known cancer driver, the mutant stem cells acted differently depending on whether they were surrounded by normal cells or other mutants. A mutated cell in a crowd of normal cells tipped toward renewal, multiplying itself to create a small and growing cluster of mutant stem cells. But once the mutated cluster grew large enough that cells in the interior found themselves surrounded by mutant brethren, they flipped to differentiation, halting the spread of mutated cells.

Basic tissue architecture — and basic math — appear to be controlling this switch: How big is the cluster's diameter?

"There's a consistent battle between the pro-renewal edge and the differentiating middle," Beronja said. "When [the cluster] reaches a point where they match each other, it stabilizes and locks into place."

The balance between the two forces prevents the cluster from growing further.

Beronja hopes that his work could form the basis for cancer prevention or treatment someday. Cancer cells often “rewind” their differentiation to make it easier to grow, divide and move around the body — all rarely done by most types of mature, specialized cells — and certain therapies try to nudge them back to a more-differentiated state in which they’ll stay put and stop dividing.

But these therapies are aimed at cancer that is already causing problems for a patient. Beronja sees potential to develop strategies to keep cancer from starting in the first place.

He believes that cancer arises not just when cancer cells gain the right mutations, but when the tumor-suppressing, or mutation-tolerating, mechanisms in skin fail. Perhaps they’re overwhelmed by a tsunami of mutations, or perhaps they falter with age. He and his team have turned their focus to human head and neck tumors to see if they can identify and reactivate mutation-tolerating mechanisms to suppress tumor growth.

“Our goal is to manipulate these pathways,” Beronja said. “We want to find factors that should be either activated or inhibited in the context of a tumor that can alter its growth. A tumor’s renewal rate is about 0.6. To me, it seems much simpler to get it just below 0.5 than trying to kill every tumor cell in patients.”

Read more about how Hutch scientists are rethinking how cancer doesn’t happen in Part 1 of this two-part series.

This article was originally published August 15, 2022, by Hutch News. It is republished with permission.
