Plus

Resistance is futile

Closing cancer cells' escape route

By Krista Conger
Illustration by Brian Stauffer
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It was a triumph. For the first time, people with an inoperable form of the most common type of skin cancer had a drug treatment option that was well-tolerated, quick and — in many cases — almost freakishly successful. Doctors breathed a sigh of relief. No longer would their patients have to suffer.

"How often in your life do you get to have worked within a field where you finally get to test a drug that actually changes people's lives?" said Jean Tang (https://med.stanford.edu/profiles/jean-tang), MD, PhD, professor of dermatology at Stanford and first author of the 2012 study announcing the success. "We were very excited about the results."

But these doctors soon realized that for many patients the cancer returned after seemingly successful treatment with the new drug, vismodegib, which the Food and Drug Administration approved in early 2012 to treat advanced basal cell carcinoma. And when it returned, the tumors were likely to be resistant to the drug, leaving patients, once again, with limited options.

"We'd see the cancers melt away, but then the patient would return months later to say, 'Hey, doctor, what's this?'" said Anthony Oro, MD, PhD, the Eugene and Gloria Bauer Professor of Dermatology. By August 2012, Oro and professor of dermatology Anne Chang, MD, published a study showing that the tumors recurred in about 1 in 5 patients within a year of treatment.

The researchers realized that the new tumors were somehow evading the drug that seemed like a magic bullet the first time it was used. But how? Recently, Oro and graduate student Catherine Yao learned that, like master spies, the cells dodge the treatment by shedding their identities to become a different type of cell entirely — one that is no longer susceptible to vismodegib.

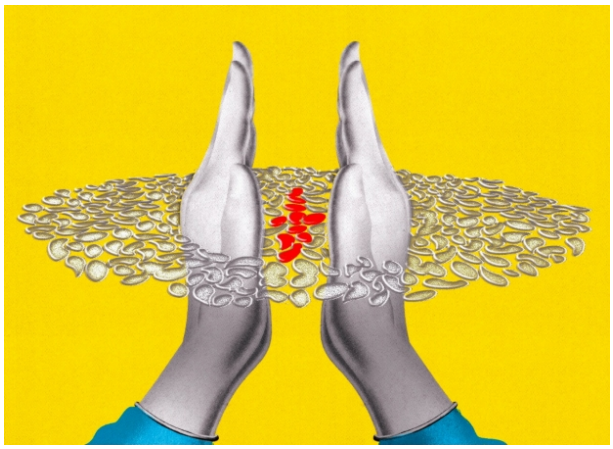

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Illustration by Brian Stauffer

The finding is the culmination of a research odyssey involving high school science fairs, an ailing father and years of study of the inner workings of mutant fruit fly embryos. But it could one day change the lives of people facing a deadly cancer.

“Finally, we’ve shown in great detail the escape route these cells have been using, and how it mirrors normal development,” Oro said. “If we can convince the drug-sensitive cancer cells to ‘stay in their lane’ by making it impossible for them to switch identities, or forcing them to switch back, we could transform the treatment of this disease.”

Understanding mutations of basal cell carcinomas

Millions of people each year are diagnosed with basal cell carcinomas, which occur primarily on sun-exposed skin. For the most part, the cancers are highly curable; they can be surgically removed or killed with radiation. But they become much more difficult to treat if they burrow deep into the surrounding tissues or spread to other parts of the body.

Basal cell carcinomas are unique among cancers because their growth relies almost exclusively on a cellular signaling system called the hedgehog pathway. Like a Rube Goldberg machine, in which a ball rolling down a track triggers weights to fall, dominoes to topple and pendulums to swing, cellular pathways comprise several successive steps that deliver a message or a signal from one location — often outside of the cell — to another — often the cell’s nucleus.

Each step triggers the next so a cell can respond quickly and efficiently to changes in its environment. Vismodegib helps stop basal cell carcinomas by blocking an early step in the hedgehog pathway.

Understanding how the hedgehog pathway works and connecting it to human cancer has been a decadeslong effort for researchers like Oro. The backstory of vismodegib’s initial success has its roots in studies of the earliest developmental stages of the tiny fruit fly called Drosophila.

Developmental biologists wondered how the fly embryo knows how to properly pattern itself during development — neatly ordering head, abdominal and thorax segments before adorning them with perfectly placed antennae, legs and wings.

Without some kind of master regulators, they reasoned, you’d be just as likely to end up with a wonky Mr. Potato Head of the fly kingdom rather than the perennial pest that hovers around many of our kitchen counters and compost piles.

By the early 1980s, the researchers had identified more than 50 genes that, when mutated, disrupted the development of the embryo in macabre ways, including adding an extra set of wings, legs and antennae that sprouted from places they didn’t belong. Hedgehog was one of these genes, so named because mutant embryos had a spiny, hedgehog-like appearance.

From science fairs to hedgehogs

Oro's interest in dermatology solidified in 1984, after his father had suffered for years from a terminal slow-growing salivary gland cancer. While completing a summer internship at the National Cancer Institute, the then Stanford undergraduate resolved to apply for an MD/PhD program to study the molecular basis of the disease that was killing his father.

He had a good background for it. In the early 1980s, Oro was among the first students at Gompers Preparatory Academy, a newly designated math and science magnet school in San Diego. The school became nationally known for the strength of its teaching and its many national awards in academic competitions.

"We didn't have a sports team, but science fair was like sports for us," Oro said. "It was a very big deal at the school."

One of Oro's science fair projects used a technique called an Ames assay that tests whether certain chemicals are likely to cause mutations in the DNA of bacteria. Rather than studying the impact of a chemical, Oro examined the potential of different kinds of UV light to cause mutations, with the goal of assessing how the loss of the ozone layer was likely to affect skin cancer rates.

"I didn't realize at the time, but I guess I was a budding dermatologist even then," Oro remarked.

Oro completed his medical and graduate school training at UC San Diego, where he studied how proteins in the nucleus of Drosophila affect pattern formation in the fly embryo. (At the time, the fields of developmental biology and cancer were beginning to merge as it became clear that the patterns of cell division and organization in a tumor often mirror those that occur during normal embryogenesis.)

He returned to Stanford in 1993 as a postdoctoral scholar in the laboratory of developmental biologist Matthew Scott (https://med.stanford.edu/profiles/matthew-scott), PhD. In 1984, Scott was one of several researchers to discover an important class of genes that shared a common DNA sequence called a homeobox. These genes carried the instructions for proteins that, when activated by the presence of the master regulators, turned on or off large swaths of genes involved in development and patterning in Drosophila.

"Once you start to understand how these regulators can flip a switch to control cell fate during development, it seems not so crazy that an adult cancer cell can also change fate," Oro said. "It happens a lot in Drosophila genetics."

The timing was fortuitous. That same year, researchers identified a hedgehog-like protein in humans, called sonic hedgehog, and Scott and Oro focused on understanding the hedgehog pathway in vertebrates, which is critically important during fetal development but rarely active in healthy adult cells.

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In 1997, the researchers showed that mutations in a protein in the pathway called patched are associated with developmental defects and human cancers, including basal cell carcinomas — a revelation that gave researchers around the world a new target for new cancer therapies.

Shortly afterward, Oro started his own lab, focusing on skin cancers as a model to learn more about how cancer cells change and grow.

“Just like in fruit fly embryos, where the effects of mutations are easy to spot, skin cancers are highly visible and easily biopsied, making them a great model to study broad themes in cancer development and evolution,” Oro said.

Discoveries by Oro and Scott and subsequent clinical trials at Stanford and elsewhere led to the 2012 approval of vismodegib, which inhibits another protein in the hedgehog pathway called smoothened. Though the pathway is inactive in most adult tissues, it is always active in basal cell carcinomas, sending constant signals to the cancer cell to divide relentlessly. Inhibiting smoothened acts as an emergency brake in the pathway, stopping the growth of the cancer.

“Tony and Matt really established the hedgehog pathway as the central pathogenic pathway in basal cell carcinomas and focused attention on the possibility of perturbing this process to treat these types of cancers,” said Paul Khavari (https://profiles.stanford.edu/paul-khavari), MD, PhD, the Carl J. Herzog Professor in Dermatology and chair of the department.

Rooting out cancer in hair follicles

Surprisingly, although basal cell carcinomas are skin cancers, they actually arise from stem cells in the hair follicles that pepper most of skin’s surface. Although the hedgehog pathway is inactive in most adult tissues, stem cells in the follicle toggle it on and off to regulate the rapid cycles of cell division and rest that drive the hair growth and loss that plague bathtub drains. Basal cell carcinomas arise when the pathway is always on.

Sometimes these cancers, Oro and Yao found, switch their careers in a phenomenon known as cellular plasticity, or the ability of one cell to morph into a related but different type of cell. This type of cellular transfiguration, akin to switching careers from a plumber to a carpenter or from a lawyer to a real estate agent, is one way that cancer cells evolve and change to promote metastasis or drug resistance.

Oro and Yao found that these basal cell carcinoma cells sometimes slide sideways along a common developmental pathway to more closely resemble another type of cell in the hair follicle called transit-amplifying cells. Normally, short-lived transit-amplifying cells die soon after secreting signaling factors to coordinate the growth and development of other cells inside and outside of the follicle. But the cancer cells that have assumed their identity continue to divide.

“Cancer biologists might say the cells want to hedge their bets while staying alive. If you can diversify your portfolio so that a subset of

your cells can withstand the treatment, you're golden."

Although, like the stem cells, transit-amplifying cells and their cancerous doppelgangers also rely on the hedgehog pathway for their growth, they activate it differently. In the Rube Goldberg analogy, it's as if they use a secret door to introduce a new ball below the emergency brake thrown by vismodegib — rendering the drug ineffective.

"Here, we can actually see cancer cells changing lanes or fates," Oro said. "We give vismodegib, which inhibits smoothened activity, but the cancer doesn't die. Instead it switches to a different type of cell that uses an alternative to the standard hedgehog signaling system. This allows them to live even though the upstream portion of the pathway is being blocked."

After twirling a developmental cloak to evade vismodegib, the cancer cells exist in a murky limbo between 'normal' basal cell carcinomas and true transit-amplifying cells.

"Somewhere along the normal differentiation pathway, the differentiation program is hijacked by the cancer, which uses existing pathways in new ways to drive drug resistance," Yao said.

The researchers soon discovered that this sneaky sidestep isn't rare. In fact, the resistant cells have a unique pattern of proteins on their surface, which allows researchers to identify them in tissue samples from patients with basal cell carcinoma who came to Stanford for treatment. Also, the transformation isn't always an evasive maneuver sparked by exposure to vismodegib or other smoothened inhibitors.

"Sometimes these cancers just change spontaneously," Oro said. "Some untreated samples from patients have no resistant cells in their tumors, while other patients come to us with nearly half their tumor cells already resistant to vismodegib."

Why? "Cancer biologists might say the cells want to hedge their bets while staying alive," Oro said. "If you can diversify your portfolio so that a subset of your cells can withstand the treatment, you're golden."

After working out the minutiae of the cells' drug resistance, Yao showed that simultaneously blocking smoothened with vismodegib and another protein called AP-1 dramatically decreased hedgehog pathway activity in tissue samples collected from people with basal cell carcinoma.

The finding suggests that a combination treatment targeting both arms of the pathway could be significantly more effective than inhibiting only smoothened, and it might even head off the transformation of drug-sensitive basal carcinoma cells into their resistant counterparts — in effect telling them to stay in their own lane — or re-sensitize resistant cells to vismodegib. Either would be a boon to patient care.

'Stay in your lane' combination therapy

Yao and Oro published their results in *Nature Communications* in October, and they are eager to translate their findings into patient care.

"Fortunately, we have here at Stanford a talented team of clinicians, translational scientists and basic scientists, as well as amazingly generous clinic patients who donate their samples for research," Oro said. "In particular, Sumaira Aasi, MD, who is the director of Mohs and Dermatologic Surgery, and dermatology faculty members Kavita Sarin, MD, PhD; Anne Chang, MD; and Jean Tang also lead their own independent research efforts."

Yao and Oro envision a future in which a patient's basal cell carcinoma could be evaluated for the cellular markers that indicated probable resistance to vismodegib and their treatment tailored to the results: treatment with a smoothened inhibitor like vismodegib alone if it is likely to be sensitive, or with a combination therapy including an AP-1 inhibitor if the cells have already begun to change lanes.

> *" ... If the cancer changes, well, those drugs aren't going to work. It's like trying to shoot a duck, but the duck changes into some other animal during the hunt. It's time for a different kind of weapon."*

Interestingly, the implications of their finding could spread beyond basal cell carcinomas, including to the salivary gland tumor that eventually killed Oro's father.

"All skin cancers are a type of cancer known as an adenocarcinoma," Oro said, "as are cancers of the lung, pancreas, colon and salivary gland. Preliminary research indicates that we see the same cell surface markers delineating sensitivity or resistance on those cancers as well, and that they likely follow the same principle of lane-switching to evade treatment."

As with the studies that led to the approval of vismodegib, Oro and his collaborators are well-suited to move the findings into the clinic because they straddle the chasm that often separates basic science and clinical know-how.

"All the faculty members in the dermatology department are also practicing clinicians. We all speak the same language of medicine and of basic science; there's no translator needed," said Khavari.

"This accelerates every step of the process. Tony not only made the initial observations in the laboratory but he deeply understands the clinical manifestations of the disease and what's needed to bring these types of advances into the clinic. This kind of work will provide additional options for treatment, which will be invaluable to our patients."

Oro knows what it's like to tell a patient there are no other options for their cancer. It keeps him hunting for new strategies.

"As a clinician, you might look up how to treat a specific kind of cancer and you use the recommended drugs. But if the cancer changes, well, those drugs aren't going to work," Oro said. "It's like trying to shoot a duck, but the duck changes into some other animal during the hunt. It's time for a different kind of weapon."

Latest information on COVID-19 (https://med.stanford.edu/covid19.html)

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ADDITIONAL READING

(https://stanmed.stanford.edu/2020issue2/covid-maldonado-leader-stanford.html)

(HTTPS://STANMED.STANFORD.EDU/2020ISSUE2/COVID-MALDONADO-LEADER-STANFORD.HTML)

'I've trained my whole life for this' (https://stanmed.stanford.edu/2020issue2/covid-maldonado-leader-stanford.html)

Stanford Medicine's infectious disease epidemiologist Bonnie Maldonado is tapping her years of experience fighting deadly viruses to foster focus and calm amid the COVID-19 chaos.

(https://stanmed.stanford.edu/2020issue2/how-coronavirus-destroys-cells-treatments.html)

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The invader (https://stanmed.stanford.edu/2020issue2/how-coronavirus-destroys-cells-treatments.html)

On a mission to survive, the coronavirus leaves behind a trail of destruction. Here's how it works and what an army of scientists is doing to try to end its rampage.

(HTTPS://STANMED.STANFORD.EDU/2020ISSUE1/REIMAGINING-CANCER-SURVIVAL-CARE-ADVANCES.HTML)

Reimagining survival (https://stanmed.stanford.edu/2020issue1/reimagining-cancer-survival-care-advances.html)

Cancer is often no longer necessarily a death sentence, thanks to advances in innovative diagnostics and treatments.

(HTTPS://STANMED.STANFORD.EDU/2019FALL/NEW-STANFORD-HOSPITAL.HTML)

Tomorrow's hospital today (https://stanmed.stanford.edu/2019fall/new-stanford-hospital.html)

Advanced technology and a design that puts healing and well-being first come together at the new Stanford Hospital — built to serve the community 100 years into the future.