FRANK

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Photo by Stewart Marcano

THE BRAIN TUMOR DECIPHERER

His sustained exploration of the signaling networks, communications and genetic idiosyncrasies of brain cancer cells is yielding valuable clues to new therapies.

Inspiration loves a change of scenery. This may be why it visited Frank Furnari once, roughly a decade ago, during an afternoon stroll at the gardens in San Diego's Balboa Park.

Working out of Web Cavenee's lab at Ludwig San Diego, Furnari had been picking apart how the mutant receptor EGFRvIII drives the brain cancer glioblastoma multiforme (GBM). Though a more potent engine of cell proliferation than its unmutated "wild-type" counterpart, EGFRvIII is typically found only on a minority of cells in any given GBM tumor. This is puzzling because rapidly growing EGFRvIII cells should take over the whole tumor. Eyeing the lush vegetation in the garden, Furnari found himself pondering ecological interactions. "I'm looking at these trees and thinking, 'The tree is helping the orchid grow, and there's no damage to the tree, even though the orchid is thriving, embedded in the tree,'" he recalls. "Then I got to thinking how if you mix crops, you get a much higher yield in your harvest. I wondered, 'Could something like this be happening in the tumors?' "

He was onto something. Furnari and his colleagues reported in *Genes & Development* in 2010 that signals from EGFRvIII prompt cells to secrete a factor named interleukin-6 (IL-6), which fuels the proliferation of both cell types, keeping their proportions within the tumor steady. But do cells expressing EGFRvIII also protect their wild-type cousins from therapy—much as plant diversity shields crops from weeds and pests?

In 2017, Furnari and his colleagues reported in *Genes & Development* that the answer, once again, is yes and that the process works by driving the expression of a protein that prevents the suicide of GBM cells deprived of the receptor's signals. The team's findings have opened a novel strategy for treating

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GBM, a currently incurable cancer that typically causes death within 14 months of diagnosis. In another study published in 2017, Furnari and his colleagues showed how GBM cells lacking the tumor suppressor PTEN can, paradoxically, be killed by disrupting the activity of a second tumor suppressor named DAXX. This effect, known as synthetic lethality, illustrates a novel and actionable approach to devising new drugs for GBM therapy.

A work ethic

Furnari grew up in Queens, New York, in an apartment above his father's butcher shop. "He would go down to the docks in the wee hours and bring back these huge sides of beef," Furnari recalls. "It was a hard life."

It got much harder, and for the whole family, when Furnari was eight years old, after his father was so disabled by a heart attack that he could no longer work. Furnari's mother, until then a homemaker, found a fulltime job as a bookkeeper to keep the family afloat. "The dynamics of our household completely changed," says Furnari. "Mom became the breadwinner. From our house in Queens, she would take buses and trains into Manhattan every day, come back in the evening and then do all the things that women did at home at the time."

The parental work ethic rubbed off on Furnari, who ran a newspaper route starting at 12 and held various jobs throughout high school. At Hofstra University, he majored in biology, minored in biochemistry and worked six to seven hours a night as a technician in a toxicology lab. After obtaining his bachelor's degree in 1985, he worked for two years as a technician in the laboratory of John Mendelsohn, who was then the chairman of the Department of Medicine at Memorial Sloan Kettering Cancer Center, in New York.

It was here that Furnari first encountered the EGF receptor while working on an antibody that targeted the protein as a potential cancer therapy. Furnari developed a fascination with cancer research that led him to begin graduate school at the University of North Carolina at Chapel Hill, where Joseph Pagano—an expert on DNA tumor viruses—was his adviser.

Furnari worked on the Epstein-Barr virus—which causes mononucleosis and is linked to nasopharyngeal carcinoma and Burkitt's lymphoma—studying how its cancer-promoting genes resemble human oncogenes. "That was the beginning of doing what I wanted to do," he says. "I wanted to study human diseases. In particular, cancer."

As his graduate work wound to a close in 1993, Furnari became interested in tumor suppressor genes, then a red-hot field. A faculty member got him in touch with Cavenee, who was opening a new Ludwig Branch at the University of California, San Diego (UCSD). Cavenee had started a GBM research program in his lab, which Furnari

Photo by Stewart Marcano

joined, focusing on the EGF receptor and, later, PTEN. His research was the first to demonstrate that restoring PTEN function in GBM tumors in which the gene was mutated suppressed their growth.

Tapping the crosstalk

Over the next 15 years, Furnari worked as section head in Cavenee's lab, becoming a tenured professor in the Department of Pathology at UCSD in 2011 and a Member of the Ludwig Institute in 2016. His research over those years continued to explore EGF receptor signaling and PTEN function and dysfunction in glioblastoma.

After the GBM genome was sequenced in 2008, it was clear that extreme genetic diversity is something of a hallmark of the tumors. This diversity is reflected in the counterintuitive distribution of EGFRvIII-expressing cells in GBM tumors that had so puzzled Furnari and led to his moment at the botanical garden.

In the 2017 *Genes & Development* paper Furnari and his colleagues reported that the IL-6 secreted in response to EGFRvIII signaling results in the activation of a nuclear factor. This factor, in concert with a protein named BRD4, boosts the expression of survivin, which saves cancer cells from death by EGF receptor inhibition. Silencing survivin expression with an experimental inhibitor of BRD4 restored sensitivity to EGF receptor inhibitors in both EGFRvIII and wild-type cells and extended the survival of mice bearing GBM tumors.

"Perhaps we can leverage the GBM tumor's heterogeneity for therapy if we can understand how interactions between genetically diverse tumor cells lead to the use of common signaling pathways that are important to survival," says Furnari. "That's the next phaseof this project."

An induced vulnerability

The study published in *Nature Communications* in 2017 stemmed from the observation that about 40% of GBM tumors sport deletions of

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PTEN, which would make them resistant to EGF receptor inhibitors. This is because PTEN inactivates a pathway involved in the EGF receptor's signaling cascade—the PI3 kinase pathway—that would, in its absence, be constantly active.

Furnari and postdoc Jorge Benitez wondered whether cells with the PTEN deletion might be susceptible to synthetic lethality. "Are there signaling pathways or other growth-promoting mechanisms in cells that are only essential when PTEN is deleted?" Furnari recalls thinking. "Something that creates a vulnerability in PTEN-deleted cells?"

In exploring that possibility, they discovered a three-way interaction between PTEN, a protein involved in packaging DNA known as histone 3.3 (H3.3), and DAXX, a so-called chaperone protein, which helps guide the attachment of H3.3 to DNA.

H3.3, however, is no ordinary packager of DNA. It also appears to play a role in suppressing the expression of cancer genes. Knocking out DAXX in cells lacking PTEN, they discovered, silenced the same cancer genes suppressed by PTEN. "It was as if we'd restored PTEN to these cells," says Furnari. "In the absence of DAXX, histone 3.3 was able to repress the activation of these oncogenes, and the effect was only seen in cells that lacked PTEN. It was a great example of synthetic lethality."

The researchers reported that PTEN works against cancer in part by boosting the deposition of DAXX and H3.3 onto chromatin—the general term for DNA and its protein packaging. They proposed that in the absence of PTEN, DAXX and chromatin compete for H3.3, freeing up cancer genes for expression.

But when both PTEN and DAXX are deleted, H3.3 is once again free to bind to the chromatin. In support of that model, they found that if either PTEN or DAXX was

Photo by Stewart Marcano

eliminated, tumors continued to grow in a mouse model of GBM. But when both were deleted, that growth slowed considerably.

Furnari and his team are now collaborating with Geoff Wahl at La Jolla's Salk Institute to find small molecules and protein fragments that disrupt DAXX's interaction with H3.3. Such molecules could be useful for the treatment of GBM tumors that have PTEN mutations.

Today Furnari is increasingly turning his attention to developing better animal models for GBM using novel genome editing techniques. So far, he says, his team's models have faithfully recapitulated the mutations and biology of various subtypes of GBM. "We're very excited by this program because we think we can make just about any tumor type given the right combination of mutations that we dial in using genome editing."

Those models will no doubt be put to excellent use.

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