Paul Mischel

Photo by Stewart Marcano

CANCER'S SUBVERSIVE INQUISITOR

Paul Mischel's long exploration of the cancer cell's adaptability led him to one startling discovery about cancer genes and another about a brain tumor's dependency on stolen cholesterol.

About a week after Paul Mischel arrived at Ludwig San Diego in 2012, Andy Shiau and Tim Gahman of Ludwig's Small Molecule Discovery Program stopped by his office bearing gifts. Two gifts, to be precise.

One was a brain-shaped lollipop, the other a vial of LXR-623, an experimental drug once fielded in clinical trials for heart disease but dropped because people on it tended, of all things, to lose track of time. "They're fantastic colleagues," Mischel says of Shiau and Gahman, who had worked on cholesterol drugs in the private sector before joining Ludwig. "They had scanned the literature carefully and recognized this critical opportunity in a molecule that wasn't even meant to treat cancer."

In 2016, Mischel and a colleague at The Scripps Research Institute published a study they'd led that validated Shiau and Gahman's instincts. The researchers reported in *Cancer Cell* that LXR-623 crosses the blood-brain barrier—the achievement symbolized by the lollipop, and manifested in the loss of time—where it selectively kills cells of the aggressive and preternaturally drug-resistant brain cancer glioblastoma multiforme (GBM). Their paper describes how the drug sabotages a key metabolic adaptation of GBM cells, and shows that therapies that target novel vulnerabilities in cancer cells might be found outside the traditional cancer drug pipeline.

Mischel was far from done for the year. Working with colleagues at the University of California, San Diego, he also completed a long-running study on an entirely different phenomenon. The paper, published in *Nature* in early 2017, upended a fundamental assumption of cancer biology. It reported that, across a broad spectrum of tumor types, cancer genes are primarily located not on chromosomes, as had long been assumed, but on circular fragments of extrachromosomal DNA (ecDNA). The distinction is not academic. Mischel and his colleagues found that oncogenes located on ecDNA drive tumor evolution and drug resistance far more potently than their chromosomal counterparts. Their discovery fundamentally alters how researchers will now regard tumor evolution and has implications for the development of cancer therapies.

METABOLIC DEXTERITY

When Mischel, trained as a clinical pathologist and then as a scientist, set up his first laboratory at UCLA in 2001, he turned his attention to dissecting the signaling pathways that drive GBM, working with Charles Sawyers, who is today at Memorial Sloan Kettering in New York. Together, the researchers uncovered some key molecular tricks GBM cells employ to resist therapy. Yet, though the research was rewarding and productive, Mischel saw challenges ahead. "It was almost like we were chasing our

tails: We were always going to be one step behind cancer's ability to adapt and develop resistance to therapy."

The problem, he grew convinced, needed to be considered from a number of different angles, with an eye to how the GBM cell adapts to both its particular environment—the brain—and to therapy. One approach to discovering new vulnerabilities that fascinated Mischel was cellular metabolism.

"One of the most important things mutations in GBM do is change how the tumor takes up and utilizes nutrients," he says. "If we could define those changes, we might begin to understand and identify new vulnerabilities in GBM tumors."

Over the past several years, Mischel and his colleagues have shown how the signals transmitted by EGF receptor vIII (EGFRvIII), a mutant cell-surface protein that often drives the fierce proliferation of GBM cells, is linked to their metabolic control systems. His team has elucidated how its signals cascade through the GBM cell, coordinated by protein complexes known as mTORC 1 and 2, to not only fuel growth but alter the import and processing of vital nutrients that support

such growth as well. In 2015, he worked with Ludwig San Diego's Bing Ren to detail the molecular cascades by which EGFRvIII alters the chemical, or "epigenetic," modification and reading of the GBM genome to reprogram cellular metabolism.

Work previously done in Mischel's lab at UCLA had revealed that GBM tumors are exceptionally rich in cholesterol, even by the standards of the brain, which holds 20% of the body's total. Those studies also revealed that EGFRvIII was responsible for GBM's cholesterol glut, and that tumor cells import (rather than produce) vast quantities of the molecule. Indeed, blocking cholesterol import proved especially lethal to GBM cells. Why this was the case, however, remained unclear.

Mischel and his team decided to take a deeper dive into that dependency in a collaboration with the laboratory of Benjamin Cravatt of The Scripps Research Institute.

BAD CHOLESTEROL
When normal cells have enough cholesterol, they start pumping out the excess and convert some of it into molecules known as oxysterols. These molecules activate a receptor in the cell's nucleus called the liver X receptor (LXR), which turns on the genes that coordinate that process.

In 2016, Mischel, Cravatt and their colleagues reported in their *Cancer Cell* paper that GBM cells are extremely dependent on imported cholesterol because they don't make their own. They also showed that GBM cells shut down the production of oxysterols to keep the cholesterol coming. LXR-623, which short-circuits that mechanism by independently activating LXR, not only penetrates the GBM tumor but selectively kills cancer cells.

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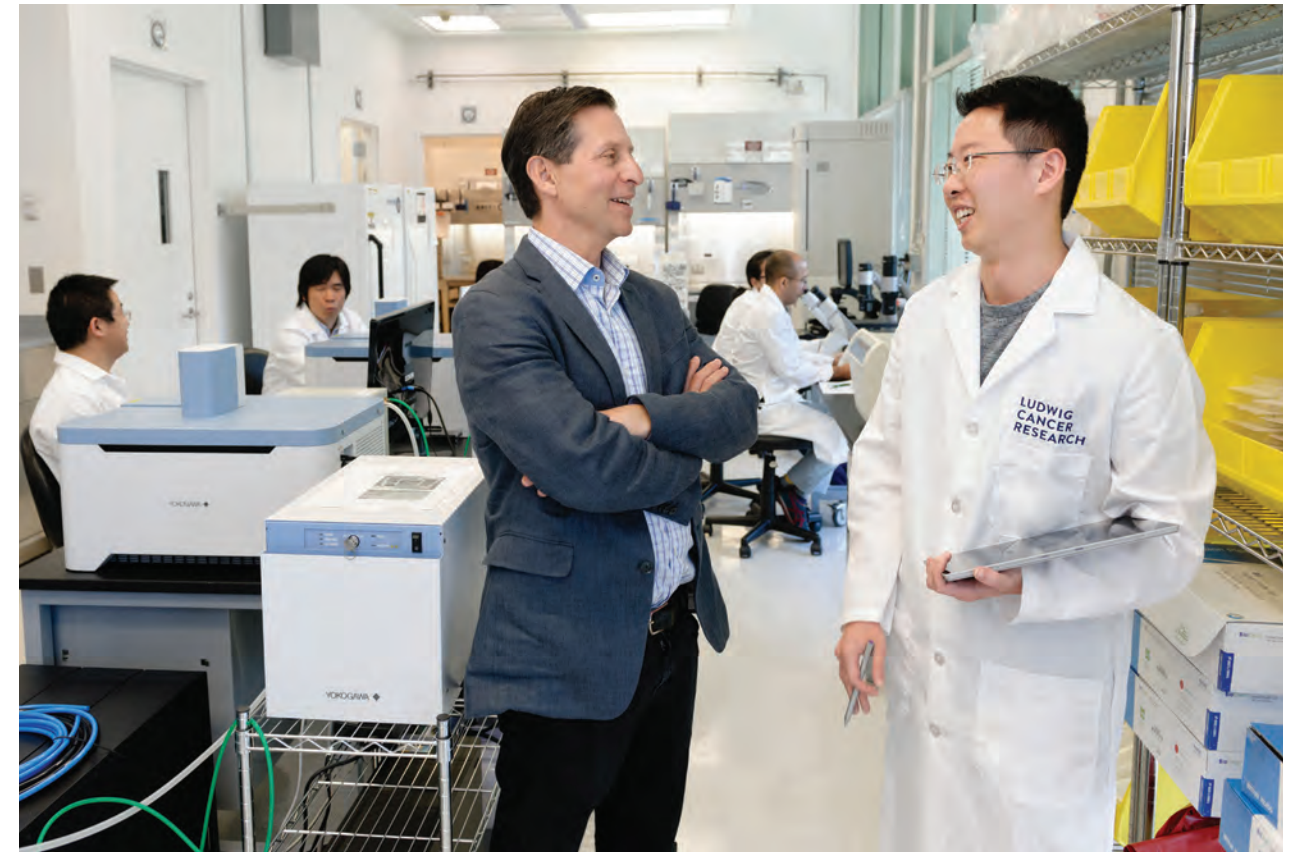
advantage of it," says Mischel. "This is a real example of the tumor adapting to scavenge resources. But it also creates a vulnerability because they switch off the stop mechanism for cholesterol import and fail to produce their own stock of the molecule. This creates a metabolic codependency, making the GBM cells vulnerable to drugs that turn that switch back on."

Mischel and his colleagues examined LXR-623's effect on GBM tumors taken from patients and implanted in mice. The drug, they showed, significantly slowed the growth of the tumors and prolonged the survival of treated mice. It did so in every GBM tumor examined and even with other types of tumors that had metastasized to the brain.

The drug's ability to cross the blood-brain barrier excites Mischel because few drugs can do that very well. This failure causes inadequate dosing, which in turn drives GBM's drug resistance.

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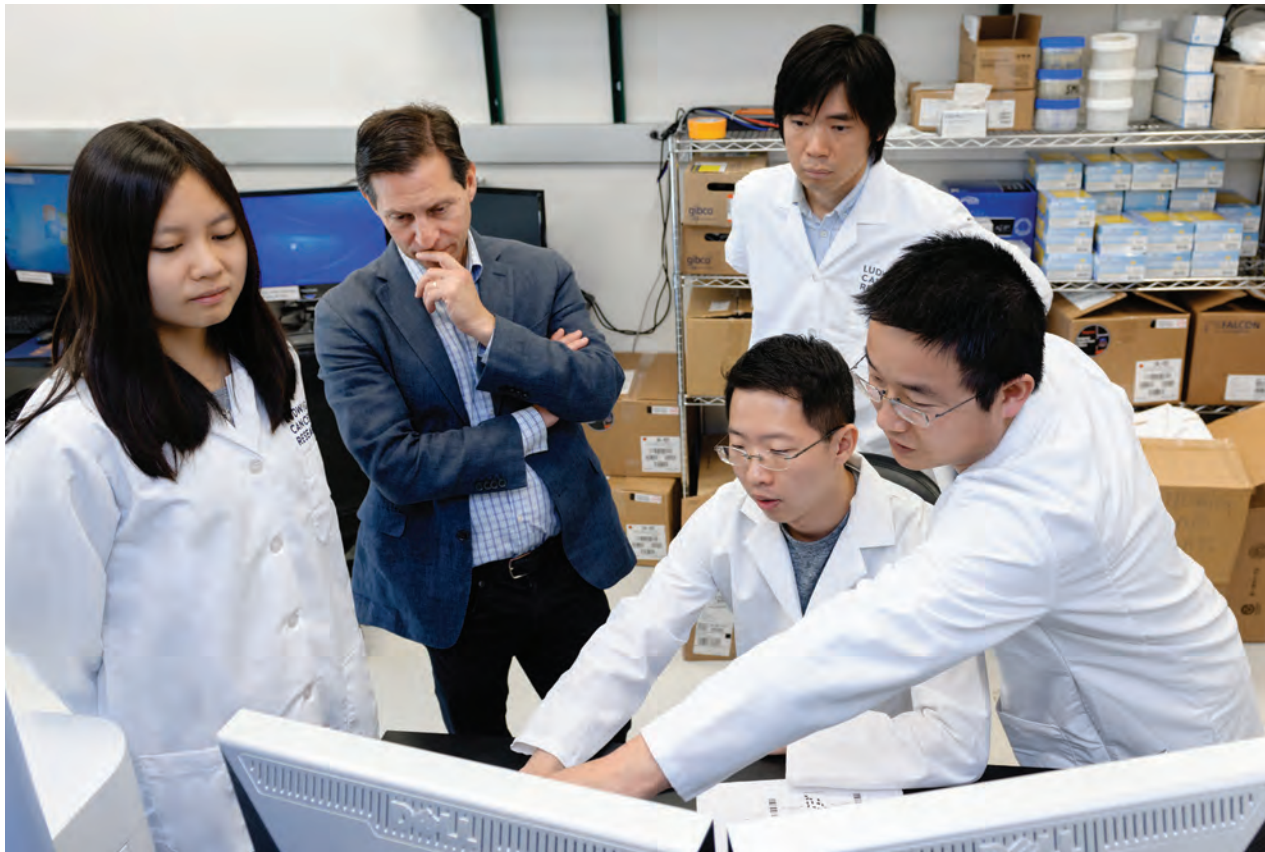
Photo by Stewart Marcano

"The important thing here is that by targeting different aspects of a tumor's adaptations, rather than just its growth, we might be able to take advantage of drugs coming from a variety of pipelines," says Mischel. "These drugs can have far more favorable pharmacological properties."

BROADER ADAPTATIONS

Along with their studies of cancer metabolism, Mischel and his team have continued a parallel and sometimes overlapping line of investigation into the tumor's many mechanisms of drug resistance. In early 2016, for example, they co-authored a paper in *Cancer Cell* with James Heath of the California Institute of Technology in which the researchers analyzed responses to therapy in individual GBM cells. They showed that the cells begin adapting their internal signaling networks to resist therapy within as little as three days of its initiation.

Such adaptations have long fascinated

Mischel. In the early years of this decade, he and his colleagues at Ludwig San Diego, Frank Furnari and Web Cavenee, were looking at how GBM tumors evolve against drugs that block EGF receptor signaling when they noticed something startling. In a paper published in 2014 in *Science*, they reported that GBM cells expressing EGFRvIII stored genes for the mutant receptor not only on their chromosomes but on circular elements of DNA, or ecDNA, as well. Strangely, when exposed to EGFR-targeting drugs, the tumors seemed to "hide" their ecDNA; when the treatment was stopped, the ecDNA would come screaming back to drive growth.

LOCATION, LOCATION, LOCATION

Conversations with other researchers who had also noticed ecDNA in cancer cells turned Mischel's interest in the phenomenon into a minor obsession. But when he scoured the scientific literature, he found that while ecDNA had been seen in tumor cells decades ago, it had long been assumed to be rare

and inconsequential. Cancer biologists had focused almost exclusively on which genes promote cancer, not where in the nucleus those genes are located. Genomics technologies had at the same time evolved along lines that favored the former type of analysis. As a consequence, nobody had really looked into the matter seriously.

Mischel decided to start looking. Led by post-doc Kristen Turner, Mischel's team applied classical cell genetics techniques and integrated them with cutting edge genomics to get a grasp of how common ecDNA might be across 17 distinct types of cancers. They found ecDNA in 40% of tumor cell lines and in nearly 90% of patient-derived models of brain tumors, but very rarely in normal cells.

"Once we saw how big an issue this was, we started thinking about the fundamental question of why," says Mischel. "Why would this actually happen? What's the benefit to a tumor of having an oncogene on ecDNA as opposed to a chromosome?"

Trouble was, given the paucity of research into ecDNA, there were no biological models in which to conduct the necessary experiments. So Mischel began working with Vineet Bafna—a computational biologist at UC San Diego introduced to him by Bing Ren—to build mathematical models of the influence ecDNA would have on tumor evolution. The researchers then vetted those predictions against the results of experiments conducted on tumor samples from patients.

They found that cancer genes are far more likely to occur on ecDNA than on chromosomes. ecDNA apparently allowed tumors to more rapidly achieve and maintain high levels of such genes. Further, ecDNA is parceled out randomly to daughter cells when a tumor cell divides, and the researchers showed that the greater the variation in their number, the more diverse the cells in a tumor.

"There's increasing evidence that cancers have a burst of genome instability, where they go from having a gradual, stepwise accumulation of mutations to all hell breaking loose ... "

"This is likely to be of great importance to the genesis of cancers, or at the very least to the changes that occur as cancers go from early stage to highly drug-resistant, late stage tumors," says Mischel. "There's increasing evidence that cancers have a burst of genome instability, where they go from having a gradual, stepwise accumulation of mutations to all hell breaking loose in their genomes."

EcDNA, Mischel observes, might be an important driver of that transformation, and he hopes next to explore the mechanisms by which it is generated. Unraveling those processes could expose new vulnerabilities in a variety of cancers—and throw open an entirely new approach to cancer drug development. ◾