A BRAIN CANCER'S DISAPPEARING DNA

Learning how a deadly tumor evades a targeted therapy suggests how it might be defeated

Cancer cells are as complex as they are cunning. To grasp their inner workings and the multifaceted malignancies they form, it is best to examine them from a variety of angles. Working together at Ludwig San Diego, Frank Furnari, Paul Mischel and Web Cavenee provide just that sort of sophisticated perspective. Each looks at tumors in a different way. Each has a distinct expertise. Each is equally intent on tackling one of the most stubborn tumors known to modern medicine: glioblastoma multiforme (GBM), a common brain cancer that leaves most newly diagnosed patients with less than two years to live.

GBM is something of a Hydra, the mythological many-headed beast who grows two new heads each time one is cut off. Conventional chemotherapy barely singes this monster. Zap it with radiation, and it grows back quickly, only now resistant to radiation. As for targeted therapies—the reputed 'smart bombs' in the oncologic arsenal: "The field has really struck out in the first clinical trials evaluating targeted drugs for glioblastoma," admits Mischel. GBM tumors have evaded single-agent, targeted therapies, particularly those delivered at suboptimal doses, by developing many drug resistance mechanisms that ensure clinical failure.

How does GBM resist the shrewdest attempts to kill it? Cavenee, director of Ludwig San Diego, believes the question is far too complex for any individual researcher to handle. "The synergy between our groups," he explains, "is personal and scientific."

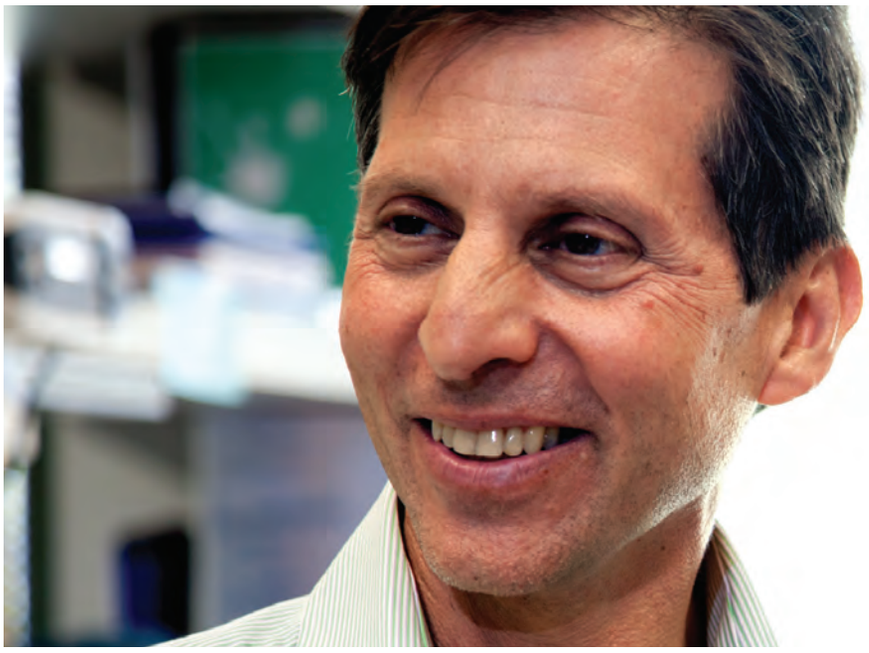
It is also paying off. Early this year, for example, the trio reported how a rogue piece of circular DNA helps GBM counteract targeted therapies, and added a layer of stunning complexity to standard models of how cancer cells resist therapy. These and other findings by the team may have set the stage for new treatment strategies not only for people with GBM but also for those with other types of cancer. "These studies will make a difference for patients in a real, substantive way," says Mischel.

SNEAKY CIRCULAR DNA

At first glance, GBM would seem an ideal candidate for targeted therapies. Most GBM tumors carry a well-known drug target, a hyperactive form of the epidermal growth factor receptor (EGFR). This protein sits on the surface of cells and drives their proliferation. Drugs called EGFR inhibitors, such as erlotinib, gefitinib and lapatinib, can shut down tumor growth and show efficacy against certain lung and breast cancers. But EGFR inhibitors have failed dismally against GBM, despite the prevalence of EGFR amplifications and mutations in GBM. The Ludwig San Diego researchers wanted to know why.

Frank Furnari, Ludwig San Diego

Web Cavenee, Ludwig San Diego



Paul Mischel, Ludwig San Diego

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WEB CAVENEE

Previous studies have shown that the gene encoding EGFR resides in a peculiar place in GBM cells—on a circular piece of DNA, separate from the chromosomal DNA that normally encodes a cell’s genes. When the researchers applied EGFR inhibitors to GBM cells in a petri dish, they found that the cells didn’t just disable expression of the gene to gain resistance. Instead, the circular DNA itself vanished. “Nobody had ever described that before,” says Furnari, referring to the disappearing act pulled off by the circular DNA. “When we saw that, we were off to the races.”

Mischel had been perfecting techniques to isolate single tumor cells and manipulate them individually. Furnari has a background in virology, and is very good at examining tiny bits of DNA in human cells. Together they examined the DNA of hundreds of individual cells taken directly from GBM patients. The team confirmed that the circular DNA’s disappearing act was no laboratory artifact: it happened in experiments performed on actual tumors taken from patients who were treated with an EGFR inhibitor. Equally surprising was their discovery that taking the drug away led to the swift reappearance of the DNA.

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That molecular game may have implications for cancer treatment. “The findings suggest a very

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PAUL MISCHEL

different dosing strategy,” says Cavenee. Cancer patients are generally treated with a moderate but sustained regimen of EGFR inhibitors. Instead, the researchers propose pulsing patients with a high dose of EGFR inhibitors to more effectively kill cancerous cells, followed by a drug holiday—a pause in the regimen—when tumors become resistant. This would permit re-emergence of the DNA encoding the target EGFR, which could then be hit once again with the EGFR-targeting therapy.

The team is now investigating whether this mechanism of hide-and-seek occurs in other types of tumors as well. “We have to look deeper,” says Cavenee. “Is this a widespread mechanism in cancer?” A glimmer of support comes from a recent study by George Demetri, director of Ludwig Harvard, and his colleagues. The work hints that gastrointestinal stromal tumor patients who have become resistant to targeted therapy with kinase inhibitors can become resensitized to the same drugs after introduction of an alternative inhibitor or even after a drug holiday. This may work even better if higher doses of the drug are pulsed periodically, as both dose and schedule appear to be important variables.

BENCH TO BEDSIDE AND BACK AGAIN

The researchers have also identified another way that GBM evades EGFR inhibitors and related drugs. They have shown that GBM cells crank up production of a molecule called PML in response to such drugs. PML obstructs the activity of the drugs. They are now gearing up to launch a clinical trial in collaboration with Ludwig’s clinical trials management group. It will test whether the combination of two drugs—arsenic trioxide, which inhibits PML, and TOR kinase inhibitors, which blunt downstream EGFR signaling—does a better job of stalling GBM.

“We use the data from the trials together with the biology we uncover in the lab to design the next iteration of clinical trials that will make a difference,” says Mischel. “That is the cornerstone of our collaborative approach.”

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