Targeted therapy triggers complex mechanism of resistance

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In order for targeted therapies against cancer to be effective, scientists need to understand upfront what related proteins in a signaling "network" makes a cancer cell resistant to a drug and selectively target them as well, say researchers at Georgetown Lombardi Comprehensive Cancer Center and Fox Chase Cancer Center.

In the September 21 issue of *Science Signaling*, the investigators discuss how cancer cells activate a network of pro-growth proteins that can bypass a molecule being therapeutically targeted. The researchers specifically found that many different genes were involved in rescuing cancer cells from treatment by different FDA-approved drugs that are designed to shut down the epidermal growth factor receptor (EGFR) - a major driver of cancer and a target of many new therapies.

This evolutionary pressure to survive is the essence of drug resistance, and the only way to treat cancer in the face of such resistance is to also disarm some of those key "rescue" genes and proteins, says study co-author Louis Weiner, MD, director of Lombardi.

"We need to be thinking about targeting a biological network, not just a single molecule," says Weiner. "The field of targeted therapy has focused enormously on the target, less on the target's signaling pathway, and almost never on the network in which this signaling pathway is embedded. That needs to change."

He adds that the study demonstrates why targeted therapies don't work at

all in many people, and function for only a short time in those who do initially respond.

To understand that, Weiner describes the EGFR signaling network as one that has dense connections among signaling proteins, feedback signaling loops, and a tendency toward protective redundancy provided by proteins that have overlapping functions.

"So any time you put pressure on cells and their dynamic signaling networks, you are inevitably challenging them to get around the problem they are experiencing," Weiner says. "We need to be smart up front and know how these cells will respond to a drug challenge, and the dominant resistance mechanisms."

Weiner began the project when he was at Fox Chase Cancer Center, conducting a phase I clinical trial of a new monoclonal antibody drug, panitumumab, in patients with metastatic colorectal cancer. They found that only about 10 percent of patients responded to the agent - as is usually the case with many targeted drugs - but that the drug was not curative. Weiner was particularly struck by one patient he said had "a remarkable, magnificent response, but then who relapsed and died."

Weiner and his co-authors began to discuss this phenomenon of initial and acquired resistance to targeted therapies, and they speculated that the reason this occurs is not due to the receptor being manipulated by the drug - in this case, EGFR. "We came to suspect that this occurs because of the network in which the EGFR signaling pathway is embedded," Weiner says. "In order to interfere with a signal coming through EGFR you have to account for many different interactions and downstream signaling."

In other words, activation of EGFR is "not a single input - single output kind of thing," he says. "The pathway is not a linear stick figure. The

output is chaotic and disparate, and there are many modifiers of this enormous complexity."

So, Weiner and the research team, which includes co-lead investigator Erica Golemis, Ph.D., and first author Igor Astsaturov, MD, PhD, of Fox Chase, embarked on an elaborate 3-year systems biology-based experimental and bioinformatics project to define all the genes and proteins in an EGFR-centered network, and then to determine which ones might activate escape signaling pathways when EGFR is targeted.

They searched databases and found 2,689 genes that encoded proteins that were linked to EGFR. To find out how these molecules function if EGFR is targeted, they chose 638 genes and targeted each gene with small interfering RNA (siRNA), which can silence gene expression. They then used a "synthetic lethal screening" technique to understand what happens to signaling proteins when an EGFR inhibitor is used.

To do this, they used a multiwell plate, in which each well was precoated with an siRNA for a specific gene the researchers wanted to target. They then placed cancer cells into each well, and added an anti-EGFR drug at a concentration that reduced the viability of the cells by about 30 percent. In this way, they could test the effect of the silenced gene on the survival of the cell, compared to treated control cells in which that gene was not silenced.

The researchers did this for 16 different colorectal cells lines and with numerous EGFR inhibitors, and found 61 genes whose knockdown sensitized the tumor cells to drug therapy. "That tells us that these 61 genes, when functional, contribute to drug resistance," Weiner says.

He adds that analyzing these 61 drug resistance genes yielded a number of surprises. One is that gene expression profiling - which is a snapshot of which genes are turned on or off at a given moment - did not identify

the genes responsible for drug resistance. Furthermore, most of the genes they identified were not mutated. "The fitness of a cancer cell is determined by the robustness of its signaling network as a whole," Weiner says. "For most solid cancers, no unique oncogenic driver has yet been identified. Instead, tumor cells undergo a process of alterations that reprogram how they function."

The other insight is that KRAS mutations are apparently not required for resistance to anti-EGFR colorectal drugs - even though patients with KRAS mutations are not given these drugs because only patients with a normal KRAS gene have been shown to benefit. "This suggests to us that KRAS is a biomarker of drug response, but is not functionally contributing to drug resistance," Weiner says. "We speculate that KRAS mutations are important in establishing a malignant network outside of the EGFR network that drives the cancer."

Weiner says that now that the research team has identified 61 genes that play some role in anti-EGFR drug resistance, they can dissect the mechanisms responsible for that resistance, and zero in on new targets for inhibition. "We can't obviously target all 61 genes, but there may be a handful of powerful genes and proteins that control nodes within this network that we can inhibit to enhance clinical therapy," he says.

And he adds that the same kind of process that he and the research team used to uncover the mechanism of drug resistance in the EGFR signaling network can be done in other powerful cancer-driving signaling networks that are also hampered by drug resistance.

"We need to embrace the complexity we know exists in treating cancer," Weiner says. "It's very complicated, but at least we now know some of the determinants of that difficulty."