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## The Vulnerable Cancer Cell

Howard Hughes Medical Institute researchers have identified many potential new drug targets for cancers long deemed “untouchable” due to the type of genetic mutation they contain. These studies are beginning to reveal new ways of attacking cancer by targeting a largely hidden network of normal genes that cancer cells rely on for survival.

Independent research teams led by Howard Hughes Medical Institute (HHMI) investigators D. Gary Gilliland of Brigham and Women’s Hospital (now senior vice president at Merck Research Laboratories) and Stephen J. Elledge at Harvard Medical School, used RNA interference (RNAi) technology to identify a host of genes that cancer cells depend on for survival. The researchers studied cells with mutations in *KRAS*, the most commonly mutated gene in human cancers.

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*KRAS*, which was discovered nearly 30 years ago, is mutated in 30 percent of human tumors, including 90 percent of pancreatic cancers, 50 percent of colon cancers, and 30 percent of non-small cell lung cancers.

“Efforts to develop drugs that inhibit oncogenic RAS proteins have been largely unsuccessful, despite the fact that *RAS* gene family members are mutated in about 30 percent of human tumors,” said Gilliland, who directs the oncology program at Merck.

More than 18 months ago, Elledge and Gilliland decided to see if they could use the powerful RNAi technology to seek out genes that *KRAS*-mutant cancer cells need for survival. Their efforts, culminating in two reports in the

May 29, 2009, issue of the journal *Cell*, have led to the identification of potentially promising drug targets: serine/threonine kinase 33 (STK33) and polo-like kinase 1 (PLK-1), as well as a host of other proteins.

“These targets represent a potential Achilles heel for tumors,” said Gilliland. “In the case of STK33, it is absolutely required for survival of cancer cells. Normal cells don’t require it.”

“The translational implications of both reports are important and immediate,” wrote Charles L. Sawyers, an HHMI investigator at Memorial Sloan-Kettering Cancer Center. Sawyers discussed the implications of the research in a *Preview* article published in the same issue of *Cell*.

Sawyers points to the identification of the two kinases as validation of the approaches taken by Elledge and Gilliland. With the dramatic clinical success of cancer drugs, such as Gleevec and dasatinib, which target rogue kinases, Sawyers says any screen that turns up new kinases is worthy of further investigation.

“The new mantra, quite simply, is that cancers bearing oncogenic mutations in a kinase are dependent on that kinase for growth and survival,” writes Sawyers in *Cell*. “With rare exception, patients with such tumors have derived significant benefit (that is, their tumors shrink) when treated with an inhibitor of that mutant kinase. The probability of success in such patients is so high that drug discovery programs can (and should) be launched when a new kinase mutation is discovered in a subset of human cancers.”

“Hopefully drugs that target non-mutant, but synthetic lethal kinases will be similarly effective,” Sawyers added.

The concept of synthetic lethality – which is part of the intellectual framework of these two studies -- has its roots in yeast genetics. Synthetic lethality is defined as a genetic interaction where the combination of mutations in two or more genes leads to cell death. For example, two different strains of yeast may each harbor a mutation that is not lethal on its own. But when both mutations are combined in a single strain of yeast, death occurs – hence the name, synthetic lethality. “Synthetic lethality is actually co-lethality,” said Elledge.

During the last few years cancer researchers have become increasingly interested in developing synthetic lethality screens as a tool for uncovering genetic dependencies in cancer cells. The rationale behind the strategy is as follows: A mutated cancer-causing gene, or oncogene, causes a cell to grow abnormally. That abnormal growth can lead to the development of a tumor. But oncogenes do not cause cancer by themselves – they depend on the activity of other genes. These genes are considered “dependents,” in the sense that the cancer cell’s survival also depends on the activity of the oncogenes and its dependent genes. Many of these so-called dependent genes are not mutated in cancer cells, but they contribute to abnormal cell growth and cancer. By using RNAi to knock down the expression of individual genes in cells bearing mutations in an oncogene, such as *KRAS*, researchers can see which gene knock-downs affect cancer cells’ viability. Gene dependencies are uncovered in cancer cells that fail to thrive.

Until recently, however, researchers simply did not have the tools to undertake a large-scale, systematic analysis to uncover genetic dependencies in mammalian cells. The discovery of RNAi a little more than a decade ago is making it possible to do genetics in mammalian cells. The cellular machinery involved in RNAi first identifies short segments of suspicious-looking RNA, and then destroys all identical copies of that RNA. The result: None of the protein that the RNA encodes for gets made.

While the natural function of RNAi is to prevent viruses from replicating inside cells and to control endogenous gene expression, scientists discovered they could exploit the process to squelch individual gene products. To do so, they introduce a short segment of RNA that looks like one of the cell’s normal genes. The RNA interference machinery grinds into action and shuts down production of the protein made from that gene.

Gilliland’s team, which included first authors, Claudia Scholl and Stefan Fröhling, as well as HHMI investigator Tyler Jacks at MIT, began their studies about two years ago. The team’s interest in leukemias informed their decision to focus on using short hairpin RNAs (shRNAs) -- single strands of RNA that fold back on themselves -- to selectively knock down the activity of serine/threonine kinases and tyrosine kinases. In recent years, kinase inhibitors have emerged as highly successful therapy for a subset of leukemias.

“We were looking at genes that we thought we could target easily with drugs,” Gilliland said. “We looked for genes that when knocked down would confer lethality to cells that were *KRAS*-mutant, but not *KRAS*-wild-type.” This approach is a particularly attractive concept in cancer research because normal cells don’t have the same dependencies on these genes. “If you find a

vulnerability conferred by another gene, you should, in theory, have a great therapeutic window because you're not going to affect normal cells," he said.

Gilliland's group began a collaboration with William C. Hahn at the RNAi Consortium at the Broad Institute to use the Broad's automated RNAi screening technology to assess about 5,000 shRNAs targeting about 1,000 human genes in a panel of eight human cancer cell lines. The shRNAs were carried in lentiviral vectors, which the researchers used to infect four cell lines carrying *KRAS* mutations and four lines carrying *KRAS*-wild-type genes.

At the top of the "hits" identified in the screen was the serine/threonine kinase, STK33, which Gilliland describes as a "totally new gene" in cancer research circles. "There are a couple of older papers describing STK33's genetic localization and exon structure, but otherwise nothing else is known about it."

That's about to change as Gilliland and his team begin to explore why STK33 represents a liability for *KRAS*-mutant cancer cells. Evidence presented in *Cell* shows that STK33 is not a part of the RAS signaling pathway, nor is it mutated in human cancer cell lines that were tested. Gilliland said his group's experiments indicate that STK33 is involved in induction of the cell death pathway in cancer cells. "We don't have all the answers yet, but STK33 is selectively required for the survival and proliferation of mutant *KRAS*-dependent cancer cells," Gilliland said.

In the experiments reported in *Cell*, Elledge and his colleagues used an RNAi technique developed by Elledge and HHMI investigator Greg Hannon at Cold Spring Harbor Laboratory. "Overall, we were asking very simple questions: What do *RAS* cells need to survive? And is it different from what normal cells need to survive?" said Elledge.

Elledge's team generated about 75,000 bits of short hairpin RNAs that can be inserted into retroviruses. When the altered retroviruses infected either normal cells or cells that differed by only a single mutation in *KRAS*, the shRNA bound to corresponding stretches of RNA in the cells, and prevented their translation into proteins.

If the shRNA knocked down production of a protein essential to keeping the cells alive, then the abundance of that particular shRNA quickly diminished as cells died. The researchers could track the identity of the shRNA – and its

corresponding gene – by using a “barcoding” method to track the diverse pool of short hairpin RNAs in parallel. In the barcoding method, every short hairpin RNA that is made carries a unique genetic tag. This tag lets the researchers track the effect of thousands of the RNAs in a single pool of cells in a single lab dish. “These are experiments a single researcher can perform in their own lab without the need for complex robotic platforms,” said Elledge.

By tracking the abundance of each shRNA from the total pool and comparing cancer cells to cells from normal tissues, Elledge and his colleague Ji Luo identified many genes that *KRAS* is dependent on. In this manner, they were able to do a genome-wide survey, uncovering many new potential drug targets, including PLK-1, STK33, and a number of proteins involved in mitosis. “It will take some time to figure this out, but RAS is clearly having some effect on an important part of mitosis,” said Elledge. “Regardless of that mechanism, it provides a vulnerability that we can attack. And fortunately there are a lot of drugs already available that have anti-mitotic properties – and we showed that some of those drugs are more toxic to the *RAS* cells.”

Furthermore, Elledge’s group found that the expression levels of some of the genes on their “hit” list correlated with patient survival. “This argues that they really do have an important role in the clinical outcomes observed in cancer patients,” said Elledge.

Sawyers thinks these two studies are an important proof-of-concept, but much more work will be needed to identify all the underlying vulnerabilities of cancer cells. “The ultimate validation of the synthetic lethal screening strategies will be evidence that patients *KRAS*-mutant tumors benefit from treatment with STK33 or PLK1 inhibitors,” Sawyers said. “Unfortunately, we won’t have that answer for many years.”