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Chemical Biology Suggests New Way to Thwart Brain Cancer

Taking advantage of a large assortment of chemical inhibitors produced by the pharmaceutical industry as potential drugs, Howard Hughes Medical Institute researchers have synthesized and characterized a panel of compounds that may lead to new treatment strategies for targeting glioblastoma, a common type of brain tumor that usually thwarts treatment. The compounds have also revealed new information about insulin signaling, and could be a powerful tool to evaluate cellular enzymes as potential targets for drug design.

The work is detailed in two papers published in the journals *Cell* and *Cancer Cell*. HHMI investigator Kevan M. Shokat at the University of California, San Francisco, is the senior author on the *Cell* paper, published on-line April 27, 2006, which describes a pharmacological map of the family of enzymes known as PI3-kinases. Zachary A. Knight, an HHMI predoctoral fellow in Shokat's lab, is the first author of the study, which was conducted in collaboration with colleagues from UCSF. The second paper, published in the May 15, 2006, issue of the journal *Cancer Cell*, describes the effects of inhibiting these kinases in glioblastoma cells. Shokat and Knight collaborated on the *Cancer Cell* paper with senior author William A. Weiss at UCSF.

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Scientists have devoted a great deal of research to kinases because they may provide the key to better understanding of a wide array of fundamental biological processes. Kinases are a huge family of enzymes that regulate intracellular communication by tagging key molecules with a small, energy-packed chemical group known as a phosphate. Their influence over processes ranging from cell growth and survival to learning and memory

makes kinases desirable targets for new drugs, and progress in this area of research depends on the careful definition of the roles of individual enzymes.

Shokat's lab focuses on ways to manipulate kinases individually to investigate their specific roles in basic biological processes and disease. Much work has been done to tease out the roles of protein kinases—those that transfer phosphate to proteins to modify their activity. But in their latest study, Shokat's group turned its attention to a family of kinases whose importance as potential drug targets has only become apparent recently.

The phosphoinositide 3-kinases (PI-3 kinases) are lipid kinases. They help transmit a variety of messages from outside a cell to its interior by attaching phosphates to lipid molecules. PI-3 kinases are critical for regulating cell growth and survival, glucose metabolism, and the immune response. One PI-3 kinase in particular, PI3-kinase alpha, is often found to be overactive in cancers of the breast, colon, stomach, and brain, and scientists suspect inhibiting this particular kinase might be an effective way to slow or halt tumor growth.

The human PI3-kinase family includes at least 15 closely related proteins, and the precise role of each one is not yet well understood. Each one has a unique pattern of regulation, expression, and lipid or protein targets - but different members of the family typically share several properties.

Many of these proteins are co-expressed in the same cells, and subsets of them are regulated in exactly the same way. They have the same binding partners, and their ATP binding pockets—where drugs bind—are absolutely conserved. Every single amino acid is identical, Shokat noted. These similarities have made it difficult for researchers to understand how specific kinases might behave differently in the first place—let alone begin to single out family members for the kinds of detailed studies that are needed to understand whether they are viable drug targets.

Shokat and his colleagues decided to tackle the problem by using a chemical approach. Scouring patent literature for compounds that pharmaceutical companies had produced to interfere with one or more PI3-kinases, the scientists synthesized a panel of about 150 inhibitors. In the past ten years, kinases have become such important drug targets that companies have invested billions of dollars into research on these drugs, and they're great compounds, Shokat said. By using compounds already known to act against members of the kinase family, he said, we bypassed the large screening effort and got to focus on the really fun part of understanding how the compounds work and learning about the biology.

After synthesizing the inhibitors, the group tested them against 55 different kinases, including all 15 members of the PI3-kinase family, to determine which ones were the most selective and the most potent. This allowed the researchers to produce a map depicting exactly which of the inhibitors could be used to study the effects of specific PI3-kinases, detailing precisely which enzyme, or, in some cases, enzymes, were affected by each.

The scientists then determined crystal structures of several of the most potent inhibitors bound to PI3K gamma in collaboration with Roger Williams' lab at the Medical Research Council in Cambridge, England. Comparing these structures allowed them to visualize how one chemical might interfere with the function of a given PI-3 kinase, but still be physically unable to affect other members of the family. This knowledge provides the first structural explanation for selective inhibition of a lipid kinase, and will likely be useful to researchers who are attempting to design molecules that target particular signaling pathways for therapeutics, Shokat said.

With their panel of inhibitors synthesized and characterized, Shokat and his colleagues began to use the molecules to explore how PI3-kinases affected physiological processes. First, they investigated how the enzymes contributed to insulin signaling. What the isoform-specific inhibitors allowed us to do was to send a signal from insulin and at the very same moment inhibit either PI3-kinase alpha, beta, delta, or gamma, and investigate the effects, Shokat said.

When the scientists inhibited PI3-kinase alpha, they completely blocked insulin signaling, preventing cells from taking up glucose. This was in contrast to earlier studies using antibody microinjection, which had suggested that PI3-kinase beta might take over in the absence of PI3-kinase alpha. That was the first major finding of these isoform-specific inhibitors, Shokat said.

They went on to show that PI3-kinase beta actually produced a low level of insulin-like signaling, even in the absence of insulin, to maintain certain essential cellular functions.

We finally found a role for this isoform, Shokat said, adding that their findings help explain why, in contrast to PI3-kinase alpha, PI3-kinase beta is never mutated in human cancers. These isoforms are identically regulated and co-expressed in every tissue, and it was confusing why cancer wouldn't find the beta and activate it. Our results suggest that beta is always on, so you can't activate it more.

Exploring another aspect of the PI3-kinases' broad reach, the scientists next investigated the ability of 20 of their most potent inhibitors to interfere with the growth of glioblastomas—brain tumors that are notoriously difficult to treat. Many of the inhibitors interfered with PI3-kinase signaling, but only one managed to prevent the cells from dividing, both in cells grown in the laboratory and in mouse tumors.

Due to their analysis of their panel of chemicals, Shokat and his colleagues knew that this particular compound inhibited two different members of the PI3-kinases: PI3-kinase alpha and a protein known as molecular target of rapamycin, or mTOR. In their studies with cultured cells and with mice, inhibitors that acted against either of these kinases individually failed to halt cancerous growth, but, Shokat said, inhibiting both targets together was highly effective.

mTOR is named for its sensitivity to an immunosuppressive drug that is now being tested as a cancer therapy. According to Shokat, There is an interesting problem with targeting just mTOR. When mTOR gets inhibited, it sends a signal back up the signaling pathway to stimulate PI3 kinase activity again. So there's a feedback loop that rapamycin induces that some people think will make the cancer worse.

The drug that we have inhibits both mTOR plus that feedback loop. The fact that it came out as the one drug in the complete set that was the most potent spoke directly to the hypothesis that mTOR is a good target, but you need to also quench the feedback loop. The results of this study suggest that simultaneously inhibiting mTOR and PI3-kinase alpha simultaneously may be a powerful way to prevent the growth of glioblastoma.

Beyond what he and his colleagues have already found about insulin signaling and glioblastoma, Shokat expects that their panel of inhibitors will be valuable for testing the validity of future pharmaceutical targets. We basically have the chemicals one needs to apply to any process where PIP3 [a product of PI3-kinase] is produced to help figure out which isoform is important and how important it is, he said. Determining how sensitive the process is to inhibition will also be essential in deciding whether a targeting a particular PI3-kinase is likely to be useful.