

AUGUST 12, 2008

## Studies Aim to Preempt Resistance to New Class of Cancer Drugs

Howard Hughes Medical Institute investigator Kevan Shokat and his colleagues at the University of California, San Francisco, have begun scouring cancer cells for potential sites of resistance against a promising new class of drugs currently under development. Their goal is to identify resistance mutations in the lab, before they arise in patients.

The team investigated drugs that target the PI3 kinase, an enzyme that frequently malfunctions and contributes to cancer. In the August 12, 2008, issue of the journal *Cancer Cell*, Shokat's group reports that many of the genetic mutations that are expected to confer resistance to these drugs cannot be tolerated by the PI3 kinase enzyme. While Shokat and his colleagues identified some resistance-causing mutations, they found that they could counter those mutations by using drugs that simultaneously inhibit the PI3 kinase and other proteins in the same signaling pathway.

---

"There are not that many single drug targets that are mutated so frequently in cancer and that are in classes of enzymes where we know how to make a drug. PI3 kinase is one of these targets and that's why it is sky high on the list of exciting new targets in cancer."

— Kevan M. Shokat

---

In healthy cells, PI3 kinase remains inactive until it is turned on by growth factors from outside the cell. Upon activation, the enzyme attaches a small chemical called a phosphate to a molecular messenger inside the cell—a lipid known as PIP<sub>2</sub>. This signals the cell to grow and divide. When PI3 kinase is allowed to spur growth unchecked, cancer can develop. Scientists recently calculated that the enzyme is one of the most frequent proteins to become activated by mutation in cancer: overactive PI3 kinase contributes to cancers of the breast, endometrium, colon, lung, brain, liver, and stomach.

There are not that many single drug targets that are mutated so frequently in cancer and that are in classes of enzymes where we know how to make a

drug, says Shokat. PI3 kinase is one of these targets and that's why it is sky high on the list of exciting new targets in cancer.

Several drugs targeting PI3 kinase have entered clinical trials to evaluate their safety, but none have yet shown effectiveness in treating cancer. Given the attention PI3 kinase is receiving, Shokat and his colleagues wanted to examine whether inhibiting the protein would quickly lead to drug resistance, thus blunting the effectiveness of potential cancer therapies.

The team designed experiments guided in part by the groundbreaking work of Howard Hughes Medical Institute investigators Brian Druker and Charles Sawyers on the molecular mechanisms of Gleevec resistance in chronic myelogenous leukemia (CML). Gleevec successfully controls CML by shutting down BCR-ABL, an enzyme that, like PI3 kinase, signals growth by attaching a phosphate molecule to a target (in this case, a protein). But when new mutations within BCR-ABL prevent Gleevec from binding, patients become resistant to further treatment. The portion of the BCR-ABL enzyme that is susceptible to these mutations is known as the gatekeeper region.

Shokat and his colleagues started by examining a location of PI3 kinase analogous to the BCR-ABL gatekeeper. They had expected that site to be particularly capable of conferring drug resistance, but instead they found that it was exquisitely sensitive: any changes made at that location effectively shut down the enzyme.

We were surprised by this result, so we said, 'Okay, if the gatekeeper in PI3 kinase is not the functional equivalent of the gatekeeper in protein kinases, let's look at some other sites, says Shokat.

Eli Zunder, a graduate student in Shokat's laboratory, identified seven other locations within the protein that might confer drug resistance if mutated. He created versions of PI3 kinase with mutations in those locations, then introduced the mutated enzymes into the common yeast *S. cerevisiae*. Yeast do not normally have PI3 kinase, and adding the enzyme strongly inhibits growth.

To investigate how the mutations affected drug sensitivity, Zunder administered five different drugs to the yeast and measured growth. When the drugs effectively shut off PI3 kinase, the yeast were able to divide and grow. If the yeast contained a drug-resistant mutant, however, the enzyme would not be shut off and the yeast would still be growth-inhibited. All drug-resistant PI3 kinase mutants identified in *S. cerevisiae* were confirmed in mammalian cells grown in lab culture.

They found that specific changes at several locations were capable of conferring moderate resistance to some of the drugs, but not to all of the drugs. In addition, some of the changes that conferred resistance to one drug actually made the enzyme more sensitive to other drugs.

What the result says is that maybe resistance will be a little bit harder to come by than in protein kinases, so that's an advantage for those trying to make

PI3-kinase drugs, says Shokat. And it may not be that hard to come up with other compounds that inhibit cancers that become resistant.

However, Shokat says it is possible that sites of resistance will develop outside the regions tested in this study. He and his collaborators are now working on a broader screen that will look at additional potential drug resistance hotspots within the protein.

The most direct implication of this work is that it teaches people that there are resistance mutations that are going to come up and you may as well try to come up with drugs against this right now, says Shokat.