

New Leads for Cancer Drugs



Emmanuelle Meuillet

Lee Wisner, technician, (left) and Margareta Berggren, laboratory manager and coordinator for the diabetes/cancer research program, maintain cancer cells under sterile hood.

Targeting a specific biochemical pathway in tumors

By Susan McGinley

Tumor cells don't play by the rules that other cells have to follow. They grow and multiply unchecked because the mechanisms that regulate cell growth and program cell death have been turned off, making the renegade cells "immortal." Cancer researchers are focusing on the role of particular enzymes involved in this biochemical malfunction to find new ways to halt or kill malignant tumors. Generally, enzymes play a key role in the signaling pathways of individual plant and animal cells.

Scientists at the University of Arizona have identified a new series of compounds that can halt the action of a specific enzyme called protein kinase B, also known as Akt, in cancer tumor cells (*see sidebar, opposite*). Their findings have been patented and will soon reach the clinical testing phase for the treatment of patients afflicted with cancer. The research is funded by the National Institute of Health and the Arizona Biomedical Research Commission.

"We're targeting a specific pathway that's out of control in many cancers," says Emmanuelle Meuillet, an assistant professor in the Department of Nutritional Sciences and the Department of Molecular and Cellular Biology. "Many scientists have tried to target the kinase domain of Akt and have been unsuccessful. Our approach is original because we're looking at Akt's PH domain instead. No one has done this before."

The "PH" in "PH domain" is an abbreviation for the longer biochemical term "pleckstrin homology" and simply refers to a region of about 110 amino acids found in 120 proteins. Though the exact function of PH domains is not known, it has been suggested that they play a role in the "membrane recruitment"

(moving proteins to a plasma membrane upon a specific signal) of proteins containing those domains, consequently enabling them to exert their effects on various signaling pathways.

Although several drugs have already been developed to inhibit Akt activity in tumors, their success has been limited. Based on the structure of the protein, Meuillet and her colleagues at the Laboratory of Cell Signaling and Cancer Therapeutics at the UA Arizona Cancer Center have figured out a new way to disable Akt by using specific compounds that bind the PH domain of Akt. Since some tumor cells rely on Akt for survival, binding to and inhibiting the PH domain renders Akt ineffective in initiating the signaling that is required for tumor cells to survive.

Finding the compounds that can bind and inhibit Akt is a painstaking process. Daruka Mahadevan and Vijay Gokhale, crystallographers and computational modelers, search through millions of compounds daily using a powerful computer database, and determine leads that bind the best to Akt PH domains. Compounds that fit the binding "pocket" on the Akt PH domain are considered for the next step, where UA chemist Eugene Mash actually synthesizes the compounds. Once made, about 100 compounds per year go to Meuillet, who tests them on mammalian cancer lines—breast, prostate, pancreatic, colon and brain.

"First we find out if the compound actually binds to the PH domain of the protein by using various biochemical techniques," Meuillet says. "Then we 'feed' it to the cells to see if the protein is inhibited. We're finding out that some of the compounds with chemical properties that look promising are actually dropping out at this stage—because of solubility or absorption, for example."

At any step in the process the researchers may have to go back to the computer database for more leads or back to synthesize new analogues. Compounds that pass the initial *in vitro* (test tube) stage are then tested in mice and in *Drosophila* fruit flies.

“We’re looking at Akt regulation and function in fruit flies,” Meuillet explains. “Instead of looking at the protein in individual cells, we’re looking at it in flies. Even though they are very small, they have conserved the entire signaling pathway networks in their cells. The advantage to the fruit fly is that it’s a whole organism, which is a better model than looking at the effects on one cell. We can see what’s happening throughout the body.”

This research is part of the UAs “Drugs on Bugs” program, designed to better understand the action mechanism of the drugs on the flies. Sylvester Moses, a graduate student in Biochemistry and Molecular and Cellular Biology (BMCB) and BMCB undergraduate Ben George are assisting with this research, along with Lee Wisner, a research scientist in Meuillet’s lab. Subsequently, the most promising compounds are then tested in pre-clinical animal trials by Garth Powis, professor-director of the Center for Targeted Therapy at MD Anderson in Houston, Texas.

“We’re hoping to identify one compound per year that has passed through all the checkpoints and has been successful in animals for anti-tumor activity,” Meuillet says. “It usually takes five to ten years—if you’re lucky—from

drug design to clinical trials.” She started this project in 2001 and is getting close with the first compound, named PX 316.

“We’re testing this new series of compounds with animals already and the next stage is to include it in a clinical study with patients. This is the ‘bench to bedside’ principle of drug design and discovery.”

“The advantage to the fruit fly is that it’s a whole organism, which is a better model than looking at the effects on one cell.”

Meuillet notes that the drug will not be a “magic pill” that will cure cancer. It will still have to be used in conjunction with classical chemotherapy and/or radiation to shrink the existing tumors.

In addition, Meuillet and her team are looking at using the Akt pathway in other studies, such as the interaction of harmful bile acids in colorectal cancer and the helpful effects of selenium in prostate cancer, among many others. She is also studying how nutrients found in the diet may influence these pathways and cause or prevent cancer.

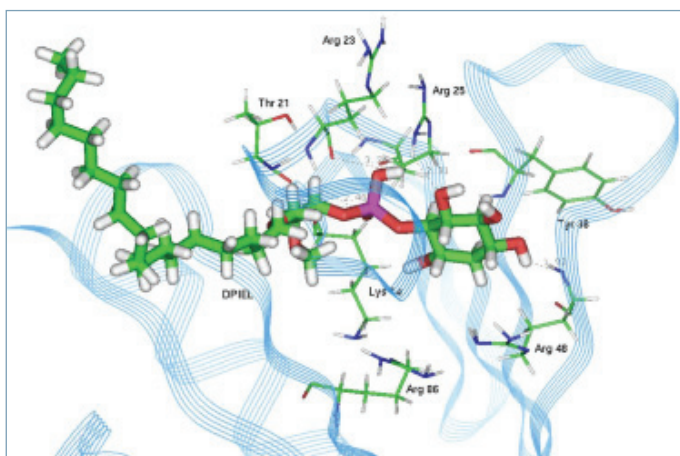
“We’re doing this in a nonprofit, academic department instead of a commercial venue, and we’re benefiting not only the general public, but students as well,” she says. “We’re looking forward to developing these technologies into a high-throughput screening facility that can handle thousands of compounds a week.” ☒



Emmanuelle Meuillet

Margareta Berggren (left) and research specialist Song Zuohe work on *Drosophila* (fruit flies) as a model for cancer and obesity. They are using a microscope to view the flies and determine their phenotype.

E.J. Meuillet, M. Berggren, R. Williams, L. Qiao, A.P. Kozikowski, G. Powis



This biochemical model shows the interaction of powerful compounds tested in Emmanuelle Meuillet’s laboratory with Akt, an enzyme that is hyperactivated in cancer cells. The compounds (in multiple colors) are inhibiting the action of Akt (blue-striped ribbon), by binding directly to the protein, and thus slowing or halting the growth of the tumor cells.

What is Akt?

Akt is an enzyme also known as protein kinase B. A kinase is a protein that transfers the phosphate group of ATP (the cell’s energy compound) to other proteins. Akt regulates many cellular events and thus is at the center of important signaling pathways that regulate cell proliferation or cell death. Problems arise when Akt is hyperactivated through the wrong signals. At that point it may cause cancer by changing the messages that regulate the growth, movement and death of cells. Tumors start growing with nothing to stop them because the mechanism in the cell that would ordinarily regulate their “life cycle” has been switched off. This phenomenon has been found in various cancer cells, including those from breast, ovarian, gastric and pancreatic tissues.

Contact

Emmanuelle Meuillet
520-626-5794
emeuillet@azcc.arizona.edu