Scientists find clues to cancer drug failure

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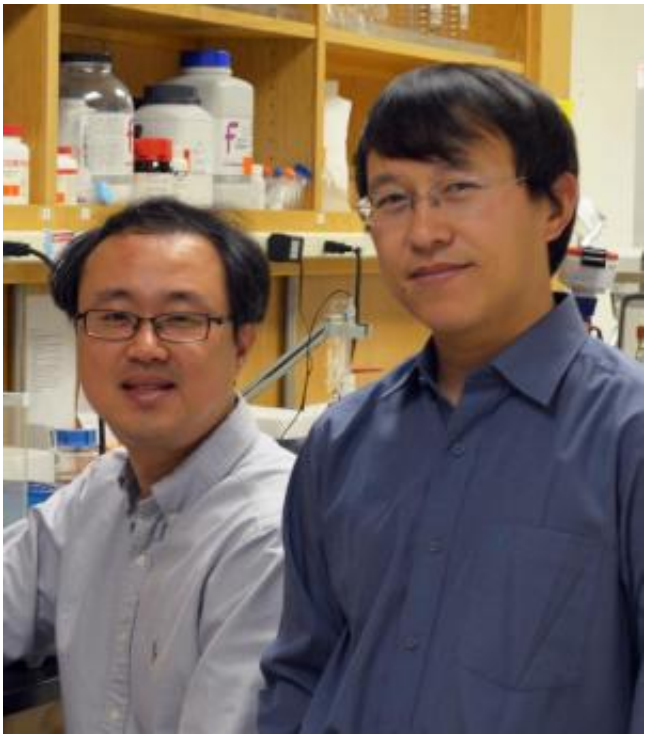

TSRI Associate Professor Qinghai Zhang (right), shown here with Research Associate Sung Chang Lee, was a senior author of both studies. Credit: Photo courtesy of The Scripps Research Institute.

Cancer patients fear the possibility that one day their cells might start rendering many different chemotherapy regimens ineffective. This phenomenon, called multidrug resistance, leads to tumors that defy treatment.

Now scientists at The Scripps Research Institute (TSRI) have published a

pair of studies showing how the primary protein responsible for multidrug chemotherapy resistance changes shape and reacts to therapeutic drugs.

"This information will help us design better molecules to inhibit or evade multidrug resistance," said TSRI Associate Professor Qinghai Zhang, a senior author of both studies.

The findings were published recently in two papers: a study in the journal *Structure* co-led by Bridget Carragher, a professor at TSRI with a joint appointment at the New York Structural Biology Center, and a paper in *Acta Crystallographica Section D* co-led by Geoffrey Chang, professor in the UC San Diego Skaggs School of Pharmacy and Pharmaceutical Sciences.

How a Protein Causes Multidrug Resistance

The proteins at work in multidrug resistance are V-shaped proteins called ABC transporters. ABC transporters are found in all kingdoms of life—from bacteria to humans. In humans, an important ABC transporter is P-glycoprotein (P-gp), which catches harmful toxins in a "binding pocket" and expels them from cells.

The problem is that in cancer patients, P-gp sometimes begins recognizing chemotherapy drugs and expelling them, too. Over time, more and more cancer cells can develop multidrug resistance, eliminating all possible treatments.

"Virtually all cancer deaths can be attributed to the failure of chemotherapy," said Zhang.

To design more effective cancer drugs, scientists would benefit from a better understanding of P-gp and how it binds to molecules.

A Better Look at Transporters

For the new *Structure* study, researchers looked at P-gp under one of TSRI's powerful electron microscopes. They also looked at MsbA, a similar transporter protein found in bacteria.

The electron microscopy (EM) work—spearheaded by a postdoctoral researcher, Arne Moeller, working in the laboratory of Carragher and former TSRI Professor Clint Potter, co-directors of the National Resource for Automated Molecular Microscopy—solved a major problem in transporter research.

Until recently, researchers could only compare images of crystal structures made from transporter proteins. These crystallography images showed single snapshots of the transporter but didn't show how the shape of the transporters could change. Using EM, however, a whole range of different conformations of the structures could be visualized, essentially capturing P-pg and MsbA in action.

The new research was also enabled by the development of new chemical tools. Previous studies were hampered by the fact that outside the cell membranes these transporter proteins turned into an unstructured mash.

"They looked like tofu," said Sung Chang Lee, a research associate in Zhang's lab at TSRI and co-first author of the *Structure* study.

In the study, the researchers used a solution of lipids and peptides to mimic natural conditions in the cell membrane. A novel chemical called beta sheet peptide, developed by the Zhang lab, was used to stabilize the protein and provide enough stability for a new perspective.

Together with EM, this technique enabled the research team to capture a series of images showing how transporter proteins change shape in

response to drug and nucleotide binding. They found that transporter proteins have an open binding pocket that constantly switches to face different sides of membranes.

"The transporter goes through many steps—it's like a machine," said Zhang.

A Closer Look at Binding

In the second study, the researchers investigated the drug binding sites of P-gp using higher-resolution X-ray crystallography.

Their findings, published in *Acta Crystallographica Section D*, show how P-gp interacts with drug-like molecules called ligands. The researchers studied crystals of the transporter bound to four different ligands to see how the transporters reacted.

The researchers found that when certain ligands bind to P-gp, they trigger local conformational changes in the transporter. Binding also increased the rate of ATP hydrolysis, which provides mechanical energy and may be the first step in the binding pocket closing process.

The team also found that ligands could bind to different areas of the transporter, leaving nearby slots open for other molecules. This suggested that it may be difficult to completely halt the drug expulsion process.

Zhang said the next step in this research is to develop molecules to evade P-gp binding.

More information: *Structure*, "Distinct Conformational Spectrum of Homologous Multidrug ABC Transporters," www.cell.com/structure/abstract/S0969-2126%2814%2900422-5

Acta Crystallographica Section D, "Snapshots of Ligand Entry, Malleable Binding, and Induced Helical Movement in P-glycoprotein," journals.iucr.org/d/issues/201 … /03/00/isscont s.html

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