Scientists find how cancer cells become resistant to chemotherapy

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A research team at the Scripps Research Institute has obtained the first glimpse of a protein that keeps certain substances, including many drugs, out of cells. The protein, called P-glycoprotein or P-gp for short, is one of the main reasons cancer cells are resistant to chemotherapy drugs. Understanding its structure may help scientists design more effective drugs.

The new research was described in the March 27, 2009, issue of the journal *Science*.

"This structure is an important advance and we hope it is just the beginning of more breakthroughs for us," says the study's senior author Geoffrey Chang, an associate professor at Scripps Research. "The structure is a nice tool for understanding how drugs are transported out of cells by P-gp and for designing drugs to evade P-gp preventing drug resistance. It's very exciting."

P-gp, a protein first identified in 1976, sits in the membrane that surrounds human cells, including those in the gut, intestine, kidney, and brain, where it functions as a gate keeper, shooing out potentially harmful agents. Problematically, P-gp not only transports substances that are harmful out of the cell, but also drugs targeted to cancer cells and HIV-infected cells, as well as some therapeutics aimed at alleviating psychiatric conditions.

"We've long known that P-glycoprotein plays a key role in multidrug

resistance in cancer patients, and this work helps us understand how the protein can act on such a wide range of compounds," said Jean Chin, Ph.D., of the National Institutes of Health's (NIH) National Institute of General Medical Sciences (NIGMS), which partially supported the work. "In the future, scientists may be able to use these crystal structures to design chemicals that block P-glycoprotein's activity and restore sensitivity to chemotherapeutic agents."

Solving the Structure

The team, which included scientists from Texas Tech University Health Sciences Center as well as Scripps Research, determined the structure of P-gp using a technique in structural biology known as x-ray crystallography, which involves making crystals of ordered arrays of protein and then blasting the frozen crystals with x-ray radiation. The atoms in the protein crystals cause the x-rays to diffract, and the scientists collect and analyze the pattern of diffraction to solve the atomic-level structure of the proteins.

"The biggest challenge was to get enough protein to purify and make crystals from it," says Stephen Aller, Ph.D., a postdoctoral fellow in Chang's laboratory and first author of the new study.

Once the scientists succeeded in performing the x-ray crystallography and solving the structure, they found that the mouse protein P-gp, which is 87 percent identical to its human counterpart, has the shape of an upside down "v" or a tipi with a large cavity inside. The cavity's interior is lined with amino acids that bind to various substances, holding them in place. The top part of the tipi resides inside the cell membrane and has two openings for substances to enter; the bottom portion sticks out inside the cell, ending in two dumbbell-shaped arms.

This overall shape is strikingly similar to that of another protein, MsbA,

that transports lipids out of bacteria. This similarity suggests that P-gp works by bringing the two dumbbell-shaped arms together on the inside of the cell and opening the closed end toward the outside of the cell, essentially reversing direction of the "v" or tipi so any substance caught inside the protein's cavity is ejected from the cell.

While the new study shows another similarity between MsbA and P-gp—both binding cavities are lined with hydrophobic amino acids—it turns out that the mammalian P-gp has many more such amino acids and a greater variety of them, including aromatic amino acids that are known to bind many different substances (substances acted on by enzymes).

"Unlike the bacterial protein, the mammalian P-gp was designed to have a wide range of substrates," says Chang. "The presence of so many hydrophobic and aromatic residues explains how this happens."

A Path to Better Drugs

The new study also produced insights by showing structures of P-gp bound to some of its substrates. Chang and Aller collaborated with Qinghai Zhang, an assistant professor at Scripps Research, who had designed several compounds that can block the function of P-gp. These compounds bind inside the P-gp cavity, preventing other substances from entering. Chang and Aller were able to obtain the structures of two of Zhang's compounds inside P-gp.

"They both go in the same cavity and bind to different amino acids, but with some overlap," says Aller. "What this tells us is that there is an extremely important core set of amino acids in P-gp that bind all substances, and there are additional amino acids for fine-tuning the binding to specific drugs."

Knowing what the P-gp binding cavity looks like and precisely where

substances bind may allow researchers design better drugs, for example by using chemistry to modify portions of that drug so that it can sneak past P-gp to get inside cells.

"[One advantage in this process is] we don't have to design brand new drugs, but rather re-design existing ones to make them work better," says Chang. "Scripps is a perfect place for these kinds of studies because there are great chemistry and biology labs here. We can easily find collaborators."

Source: The Scripps Research Institute (news : web)