Scientists determine workings of potentially useful virus

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In a study published in May 2009 issue of *PLoS Pathogens*, Manchester and her colleagues show that CPMV interacts with the mammalian protein vimentin — an interaction that scientists can now explore with the idea of using the virus to deliver "cargo," such as drugs, to tumors or other diseased tissues.

"Vimentin was not at all a likely suspect," says Kris Koudelka, a postdoctoral fellow in the Manchester lab and an author of the study. "I had to do many sets of experiments to convince myself and the team that the result was real."

Vimentin is part of the cytoskeleton, the internal scaffold that gives a cell its shape. While the vast majority of vimentin resides inside the cell, a small fraction somehow ends up on the cell's surface. This surface vimentin turns out to be the target for CPMV.

Ideal Delivery Agent

CPMV's structure, elucidated in 1999 by Scripps Research Professor John "Jack" Johnson, makes it ideal as a delivery agent. CPMV has about 300 different sites on its surface to which researchers can attach molecules. In addition, the virus, which is only harmful to plants, is compact in size—spanning only 30 nanometers in diameter (a nanometer is one billionth of a meter)—making it easy for the virus to travel throughout the body.

Manchester and colleagues showed one use for CPMV in a study published in 2006 in the scientific journal Nature Medicine, where they tagged the virus with several fluorescent dyes and injected it in mouse and chick embryos. The virus caused the blood vessels to light up by lodging itself in the endothelial cells that line the vessels' walls. "It was extremely bright and the fluorescence lasted for several days," says Manchester.

But to take full advantage of the virus's properties, Manchester and her team wanted to know more about its interactions with cells. Their first question was "What protein is the virus attaching itself to?"

"We knew this virus could be used for all kinds of applications, but we did not know exactly how it worked," says Koudelka, who first joined the Manchester lab in 2005 as a graduate student in the Scripps Research Kellogg School of Science and Technology. "It was like playing a game but with only half the instructions."

Painstaking Work

Koudelka quickly determined that the virus had an affinity for a protein weighing 54 kilodaltons (kD)—the standard unit of measurements for proteins—found on endothelial and other types of cells. The next step was to figure out the protein's identity. But pulling out a specific protein, out of the hundreds of thousands that are produced by any cell, proved to be a challenging task—one that took Koudelka more than two years to complete.

Koudelka undertook a series of painstaking steps to eliminate unwanted proteins from the mix and sequentially narrow down the pool of candidates. Reasoning that any protein CPMV bound to would reside in the cell's membrane, he separated membrane proteins from those in the cell's interior. He then removed all the proteins that were not in the

54kD size range. After a few more "elimination" steps, he fished out those proteins that stuck to CPMV. In the end, he was left with three proteins—two of them were added during the elimination procedures and are part of the virus's protective shell, or capsid, and one was the mysterious CPMV binding protein.

Using mass spectrometry—a method that involves breaking down a protein to produce a pattern of fragments that serves as the protein's fingerprint—Koudelka worked with Sunia Trauger, associate director of the Scripps Research Center for Mass Spectrometry, to demonstrate that the 54-kD protein was vimentin.

Manchester credits the success of this study to Koudelka's unfailing perseverance. "The hardest thing was to get past all the various pitfalls and test all possible caveats," she says. "You really have to be optimistic to take a project like this on as a graduate student. Kris was up to the challenge and cheerfully plowed away at it."

Exciting Implications

Knowing that surface vimentin is the receptor for CPMV will now help researchers direct the virus—and whatever cargo it carries—more precisely to its target. One possible application is to target CPMV to receptors on tumors so that it can deliver a treatment.

"We now know how the virus interacts naturally with cells and can either facilitate this interaction or mask it," explains Manchester. "For example, we can mask the sites on the virus that bind vimentin and then target the virus to a different receptor."

Another important implication of this work is that it may help find a way to prevent some infectious diseases. "Once we started to focus on vimentin, we realized that some bacterial pathogens and other viruses

also use vimentin to get inside cells," Manchester says.

Manchester and colleagues plan to study the interaction between CPMV and vimentin to determine precisely how these viruses—which unlike CPMV are harmful to human cells—invade mammalian cells, and how these viruses might be stopped.

Source: The Scripps Research Institute (news : web)