Biologists provide molecular explanation for the evolution of Tamiflu resistance

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Biologists at the California Institute of Technology have pinpointed molecular changes that helped allow the global spread of resistance to the antiviral medication Tamiflu (oseltamivir) among strains of the seasonal H1N1 flu virus.

The study—led by David Baltimore, Caltech's Robert Andrews Millikan Professor of Biology and recipient of the 1975 Nobel Prize in Physiology or Medicine, and postdoctoral scholar Jesse D. Bloom—appears in the June 4 issue of the journal *Science*.

Tamiflu and other antiviral drugs directly target viruses, unlike vaccines, which instead stimulate our body's immune system to respond to the pathogens after an infection is established.

In a flu infection, viruses bind to sialic acid on the surface of a host cell using a protein called hemagglutinin (the "H" in H1N1). The viruses then enter the cell and replicate. When the newly minted viruses exit the cell, they too bind to sialic acid. The viruses then use a protein called neuraminidase (the "N" in H1N1) to cut the sialic acid, freeing themselves to infect new cells.

This process, however, is blocked by Tamiflu, which prevents neuraminidase from cleaving the sialic acid. "It does this by binding in the 'active site' of the neuraminidase molecule, where neuraminidase normally cleaves sialic acid," Bloom says.

In general, for a virus to become resistant to Tamiflu, the neuraminidase protein has to be able to tell the difference between sialic acid (the thing it cleaves) and Tamiflu (the drug "decoy").

Such recognition is possible in viruses that have a mutation, known as H274Y, in the neuraminidase protein. The mutation swaps out one amino acid for another at a particular location on the neuraminidase protein, producing a slight conformational change in a crucial region of the protein's three-dimensional structure. "Once that happens," Bloom says, "the neuraminidase no longer strongly binds to Tamiflu, and it is still able to cleave sialic acid."

"People have known about this H274Y mutation for over a decade," he adds, "but the mutation seemed to interfere with the virus's ability to replicate and be transmitted. The molecular basis for that interference was not clear, but it seemed that the H274Y viruses weren't of great clinical significance."

However, during the 2007-2008 flu season, resistant H1N1 viruses with the H274Y mutation began cropping up all over the world. By the following year, essentially all seasonal H1N1 flu viruses suddenly were resistant to Tamiflu because of the mutation.

The only difference: They now were growing just as well as regular viruses.

"We thought it was an interesting evolutionary mystery," Bloom says. "Something happened to make the Tamiflu-resistant virus also capable of replicating and spreading like wild-type flu viruses." The question was, what?

The first step in finding out was to determine why the H274Y mutation usually hampers the growth and spread of a virus.

"Our hypothesis," Bloom says, "was that the resistance mutation was—as an incidental effect—preventing neuraminidase from reaching the cell membrane." This decreased availability of neuraminidase—the protein, remember, that cleaves newly formed viruses from their sialic-acid mooring on the host cell, allowing them to spread to infect other cells—decreased the rate of viral replication. The researchers confirmed this in cell cultures.

"Now, if you've got a second mutation that fixes this problem in H274Y mutants," Bloom says, "you'll have a virus that grows very well and is resistant to Tamiflu. And that's bad—for us, not the virus."

The researchers discovered just such a secondary mutation—two of them, in fact—in the neuraminidase gene of Tamiflu-resistant seasonal flu strains dating from the 2007-2008 flu season.

Interestingly, an examination of flu sequences showed that the two secondary mutations had cropped up before the H274Y mutation had begun to spread. The existence of these "pre-adaptive mutations," say the researchers, permitted the survival and spread of subsequent occurrences of the H274Y mutation.

Genetic changes that set the stage for later adaptations may represent a fairly common event in evolution.

"This study shows how combining an understanding of molecular mechanisms underlying evolution with the extensive sequencing data on historical isolates of influenza virus can bring about a deeper understanding of the challenge that this virus presents to the human population," says Baltimore. "Only by marshaling a wide range of available information was it possible to understand why the virus could suddenly tolerate mutations that were previously deleterious. It shows that mutations are not necessarily 'good' or 'bad,' but that their effects

may depend on the context in which they appear."

So far, the H274Y mutation has not become widespread in either the avian H5N1 influenza or the recent swine-origin influenza pandemic, although it has cropped up in isolated cases. "We hope that understanding the basis of the evolution of Tamiflu resistance in seasonal H1N1 might help in understanding what might be needed for H274Y to spread widely in these other strains as well," Bloom says.

More information: "Permissive Secondary Mutations Enable the Evolution of Influenza Oseltamivir Resistance," *Science*.

Provided by California Institute of Technology