Two-pronged protein attack could be source of SARS virulence

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Ever since the previously unknown SARS virus emerged from southern China in 2003, University of Texas Medical Branch at Galveston virologists have focused on finding the source of the pathogen's virulence — its ability to cause disease. In the 2003 epidemic, for example, between 5 and 10 percent of those who fell sick from the SARS virus died, adding up to more than 900 fatalities worldwide.

Now, UTMB researchers have uncovered what they believe could be the major factor contributing to the SARS virus' virulence: the pathogen's use of a single viral protein to weaken host cell defenses by launching a "two-pronged" attack on cellular protein-synthesis machinery.

Their results show that copies of this viral protein, known as nsp1, directly interferes with the tiny cellular machines called ribosomes, which make the proteins, such as interferon beta, that are crucial for immune defense. (If the word "ribosome" sounds familiar, it's probably because the three scientists who first determined what the miniature protein factories look like and how they function won the 2009 Nobel Prize for Chemistry.) Nsp1 is also involved in degrading the biochemical messages that are decoded by these ribosomes to produce such proteins.

"This SARS virus protein, nsp1, binds to ribosomes to inactivate them and also modifies messenger RNA molecules to make them unreadable," said UTMB professor Shinji Makino, senior author of a paper on the discovery appearing in the online edition of *Nature Structure and Molecular Biology*. "We think that this property of nsp1 could be a major

player in the virulence of SARS."

Makino and the article's other authors — postdoctoral fellows Wataru Kamitani, Cheng Huang and Kumari Lokugamage, and senior research scientist Krishna Narayanan — identified nsp1's dual effect with a series of experiments mainly done using purified nsp1 protein in a special "cell-free" system. This widely used test-tube platform, known as a "rabbit reticulocyte lysate" (RRL) system, contained only the subcellular structures and materials (ribosomes, amino acids and various control factors) that cells use to produce or "translate" proteins from messenger-RNA templates.

The researchers also developed a mutant form of the nsp1 protein that was incapable of interfering with RNA translation, employing it as an experimental control.

By measuring the outcomes produced by mixing a variety of different messenger-RNA templates with either nsp1 or mutant nsp1 in RRL, the investigators generated a strikingly detailed picture of how nsp1 interferes with ribosomes and degrades messenger RNA. Nsp1 grabs on to ribosomes, attaching to a specific part known as the 40s subunit to shut down protein production Meanwhile, the messenger RNA molecules being translated into proteins on these ribosomes are degraded by processes tied to nsp1.

"This is interesting in part because it's a new mechanism — no other known protein uses this strategy," Makino said. "But there are more practical reasons why it's important to understand viral virulence factors, particularly when you consider the potential need for treatments. There are viruses similar to SARS circulating in China, and we have no way of knowing whether this virus may come back."

Source: University of Texas Medical Branch at Galveston (news : web)