Novel toxin receptor discovered for ulcer-causing stomach pathogen

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Helicobacter pylori is one tough bug. It can survive in the human stomach, a zone with a pH somewhere between that of lemon juice and battery acid. Now researchers have discovered how an H. pylori toxin gets into cells, a feat that helps the bacterium live in one of the most inhospitable environments in the body. Their findings appear this week in *PLoS Pathogens*, a journal of the Public Library of Science.

About half of the world's population is infected with H. pylori, although most of them don't know it (most infected people have no obvious symptoms). For a percentage of the infected, however, the bacterium packs a nasty punch. H. pylori is responsible for most human cases of gastric and duodenal ulcers, and long-term infection is a significant risk factor for stomach cancer, the second leading cause of cancer death worldwide.

Researchers have tried for years to understand how the bacterium survives in the human stomach, said Steven Blanke, a University of Illinois professor in the department of microbiology and Institute for Genomic Biology and principal investigator on the study.

"Paradoxically, although H. pylori is a common resident of the human stomach, the bug is not well adapted by itself to acid," he said. "But this pathogen has several clever mechanisms for carving out a niche for itself in the stomach lining."

A protein produced by H. pylori, called vacuolating toxin A (VacA), is

an important weapon in its arsenal.

“This toxin gets into stomach epithelial cells and immune cells and changes their properties in such a way as to allow H. pylori to first gain a foothold in the stomach, and then survive over the long-term, which may be the entire lifetime of an individual,” Blanke said.

“H. pylori releases the VacA toxin in order to modify its environment,” he said.

How the toxin crossed the membrane to get into these cells was a mystery, however.

Cell membranes are composed primarily of lipids and proteins and are designed to keep things out. Some molecules can penetrate them, but most can do so only after binding to a specific membrane component, called a receptor. Receptors sometimes act as keys that open channels through a membrane, or they function as signaling molecules, communicating to other components in the cell.

Blanke’s team knew that VacA was latching on to something on the cell surface that was helping it across the membrane.

Other studies had shown that VacA bound to lipids within artificially created membranes, so graduate students Vijay Gupta and Hetal Patel screened a number of lipids for VacA binding and soon found one to which the toxin readily attached. This lipid, called sphingomyelin, is an important and abundant component of the membrane of some animal cells. (Foods such as milk, meat, fish and eggs are dietary sources of sphingomyelin.)

To be considered a receptor, a molecule must meet two criteria, Blanke said. It must bind the agent of interest (in this case VacA) to the cell

surface, and it must “confer sensitivity” to that agent. In other words, a receptor to VacA must be essential to the process by which VacA gets into a cell. If you removed the receptor, or blocked it, the toxin would lose its ability to bind or function. Prior to this study, no molecules on the membrane of human cells had been found that satisfied both criteria as a receptor.

Upon entering cells, VacA spurs the formation of giant vacuoles. These oversized membrane-bound compartments are easy to spot under a microscope and provide a useful indicator of VacA activity in the cell.

To test whether sphingomyelin was a receptor for VacA, Gupta treated cultured human cells with an enzyme that depleted the membranes of sphingomyelin. In the sphingomyelin-depleted cells, the toxin lost its ability to cross into the cells and the giant vacuoles disappeared. When he restored sphingomyelin to the same cell membranes (again, in the presence of VacA), the vacuoles returned.

“This is the first example of a bacterial virulence factor that uses sphingomyelin as a receptor,” Blanke said. “Only sphingomyelin confers sensitivity to the toxin in these cells, whereas other common membrane lipids do not.”

Sphingomyelin recently was discovered to have the ability to cluster into specialized membrane islands, or rafts, that look like raised platforms on the cell surface.

Blanke’s team found that VacA preferentially binds to and enters the cell by means of these sphingomyelin rafts.

“Our model is that these platforms serve as the entry portals for the toxin into the cell,” Blanke said. “We think that sphingomyelin is important because it seems to cluster the toxin in these portals of entry. This seems

to be absolutely essential for toxin activity."

Finding the mechanism by which the toxin gets into cells is of great interest to those hoping to treat H. pylori infection, Blanke said.

"Identifying toxin receptors is important because they are outstanding targets for new drugs to block the action of toxins on human cells," he said.

Also, because some bacterial toxins are so adept at breeching the membrane barrier to enter human cells, this work may also point the way to new strategies for sending protein-based pharmaceuticals into the cell, he said.

Source: University of Illinois at Urbana-Champaign