Fungal protein found to cross blood-brain barrier

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In a remarkable series of experiments on a fungus that causes cryptococcal meningitis, a deadly infection of the membranes that cover the spinal cord and brain, investigators at UC Davis have isolated a protein that appears to be responsible for the fungus' ability to cross from the bloodstream into the brain.

The discovery—published online June 3 in *mBio*, the open-access, peer-reviewed journal of the American Society for Microbiology—has important implications for developing a more effective treatment for *Cryptococcus neoformans*, the cause of the condition, and other brain infections, as well as for brain cancers that are difficult to treat with conventional medications.

"This study fills a significant gap in our understanding of how *C. neoformans* crosses the blood-brain barrier and causes meningitis," said Angie Gelli, associate professor of pharmacology at UC Davis and principal investigator of the study. "It is our hope that our findings will lead to improved treatment for this fungal disease as well as other diseases of the central nervous system."

Normally the brain is protected from bacterial, viral and fungal pathogens in the bloodstream by a tightly packed layer of endothelial cells lining capillaries within the central nervous system—the so-called blood-brain barrier. Relatively few organisms—and drugs that could fight brain infections or cancers—can breach this protective barrier.

The fungus studied in this research causes cryptococcal meningoencephalitis, a usually fatal brain infection that annually affects some 1 million people worldwide, most often those with an impaired immune system. People typically first develop an infection in the lungs after inhalation of the fungal spores of *C. neoformans* in soil or pigeon droppings. The pathogen then spreads to the brain and other organs.

Unique protein identified

In an effort to discover how *C. neoformans* breaches the blood-brain barrier, the investigators isolated candidate proteins from the cryptococcal cell surface. One was a previously uncharacterized metalloprotease that they named Mpr1. (A protease is an enzyme—a specialized protein—that promotes a chemical reaction; a metalloprotease contains a metal ion—in this case zinc—that is essential for its activity.) The M36 class of metalloproteases to which Mpr1 belongs is unique to fungi and does not occur in mammalian cells.

The investigators next artificially generated a strain of *C. neoformans* that lacked Mpr1 on the cell surface. Unlike the normal wild-type *C. neoformans*, the strain without Mpr1 could not cross an artificial model of the human blood-brain barrier.

They next took a strain of common baking yeast—*Saccharomyces cerevisiae*—that does not cross the blood-brain barrier and does not normally express Mpr1, and modified it to express Mpr1 on its cell surface. This strain then gained the ability to cross the blood-brain barrier model.

Investigators then infected mice with either the *C. neoformans* that lacked Mpr1 or the wild-type strain by injecting the organisms into their bloodstream. Comparing the brain pathology of mice 48 hours later, they found numerous cryptococci-filled cysts throughout the brain tissue of

mice infected with the wild-type strain; these lesions were undetectable in those infected with the strain lacking Mpr1. In another experiment, after 37 days of being infected by the inhalation route, 85 percent of the mice exposed to the wild-type *C. neoformans* had died, while all of those given the fungus without Mpr1 were alive.

"Our studies are the first clear demonstration of a specific role for a fungal protease in invading the central nervous system," said Gelli. "The details of exactly how it crosses is an important new area under investigation."

New targeted therapies possible

According to Gelli, their discovery has significant therapeutic potential via two important mechanisms. Either Mpr1—or an aspect of the mechanism by which it crosses the blood-brain barrier—could be a target of new drugs for treating meningitis caused by *C. neoformans*. In a person who develops cryptococcal lung infection, such a treatment would ideally make the fungus less likely to enter the brain and lead to a rapidly fatal meningitis.

Secondly, Mpr1 could be developed as part of a drug-delivery vehicle for brain infections and cancers. An antibiotic or cancer-fighting drug that is unable to cross the blood-brain barrier on its own could be attached to a nanoparticle containing Mpr1, allowing it to hitch a ride and deliver its goods to where it is needed.

"The biggest obstacle to treating many brain cancers and infections is getting good drugs through the blood-brain barrier," said Gelli. "If we could design an effective delivery system into the brain, the impact would be enormous for treating some of these terrible diseases."

Gelli's group is currently pursuing such a nanoparticle drug-delivery

system using Mpr1. They are also further investigating the exact molecular mechanism by which Mpr1 breaches the blood-brain barrier.

Provided by UC Davis