Disrupting mitochondrial function could improve treatment of fungal infections

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By identifying new compounds that selectively block mitochondrial respiration in pathogenic fungi, Whitehead Institute scientists have identified a potential antifungal mechanism that could enable combination therapy with fluconazole, one of today's most commonly prescribed fungal infection treatments. The approach could also prevent the development of drug resistance.

"Our research adds weight to the idea that effective antifungal drugs can target even those mitochondrial proteins that are highly conserved in humans and fungi, and that this could be a way to make a broad spectrum antifungal combination therapy that would be less susceptible to resistance," says Benjamin Vincent, a former graduate student in Whitehead Member Susan Lindquist's lab who is now a scientist at Yumanity Therapeutics.

Fungi cause bothersome diaper rashes, oral thrush, athlete's foot, and vaginal yeast infections, but they are also responsible for life-threatening infections in the immunocompromised, including patients receiving transplants, people with HIV/AIDS, cancer patients, and the elderly. Severe invasive fungal infections have a mortality rate of 30-50% and cause an estimated 1.5 million deaths worldwide annually.

Doctors rely on three main drug classes—the azoles (e.g., fluconazole), the echinocandins, and amphotericin—to treat these severe infections, but often with limited success. Many strains of pathogenic yeast, such as Candida albicans (C. albicans) can develop resistance to these drugs.

Although combining therapies is a potent method to combat drug resistance in bacteria, antifungal drugs often perform poorly when used in combination due to their complex pharmacology and antagonistic antifungal mechanisms. When used individually, current antifungal drugs can have significant toxicities that are markedly enhanced when the drugs are used in combination.

"Pharmaceutical companies are abandoning the development of antifungals," says Lindquist, who is also a Howard Hughes Medical Institute investigator and a professor of biology at MIT. "Fungi are much more similar to us than bacteria, so it is hard to find agents that attack them but not us."

To identify new potential antifungals that could be combined with fluconazole, a team of Whitehead and MIT scientists screened 300,000 compounds, selecting one with the most apparent potential—Inz-1—for further study. Their work is described online this week in the journal *Cell Chemical Biology*.

Inz-1 inhibits the growth of C. albicans in media lacking glucose but only partially impairs growth when glucose is present, indicating that Inz-1 interferes with mitochondrial function. Indeed, the researchers determined that Inz-1 targets the cytochrome B protein required for mitochondrial production of ATP. The authors then worked with synthetic chemist Jean-Baptiste Langlois in the laboratory of Stephen Buchwald in the MIT Department of Chemistry to iteratively synthesize and test analogs of Inz-1 to improve its properties. This work led to Inz-5, which exhibited dramatically improved potency and selectivity for fungal cytochrome B. Although cytochrome B is highly conserved across humans and many pathogenic fungi, including Cryptococcus neoformans, Aspergillus fumigatus, and Rhizopus oryzae, Inz-5 exploits important differences in the amino acid sequence of the protein that enable selectivity for fungi.

Because the compound is metabolized too rapidly for study in mice, the team mimicked its effects by knocking out cytochrome B in C. albicans and infecting mice with this mutant strain. Overall, the cytochrome B knock-out strain is much less virulent, and mice infected with it survive much longer than those with the wild-type strain. Curiously, the mutant yeast seems to cause more infections in the brain and central nervous system than unaltered C. albicans. Treatment with fluconazole effectively clears infection caused by this mutant, indicating that combination antifungal therapy could be highly effective when one of the agents targets mitochondrial respiration.

Not only does hitting cytochrome B disable C. albicans' virulence, but the fungus's altered mitochondrial function means that the yeast is unable to adapt to the nutrient-deprived conditions present within the host, particularly inside macrophages. Instead of punching its way out of a macrophage that has engulfed it, the yeast remains trapped and loses its fight against the immune system.

Although Inz-1's therapeutic promise is limited by its poor stability in animals, the compound proves that conserved cellular processes can be viable targets for selective antifungal therapeutics and could provide targets for effective combination antifungal therapy.

More information: "Fungal-Selective Cytochrome bc1 Inhibitor Impairs Virulence and Prevents the Evolution of Drug Resistance," *Cell Chemical Biology*, online August 11, 2016.

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