CRISPR-Cas editing of C. albicans holds promise for overcoming deadly fungal infections

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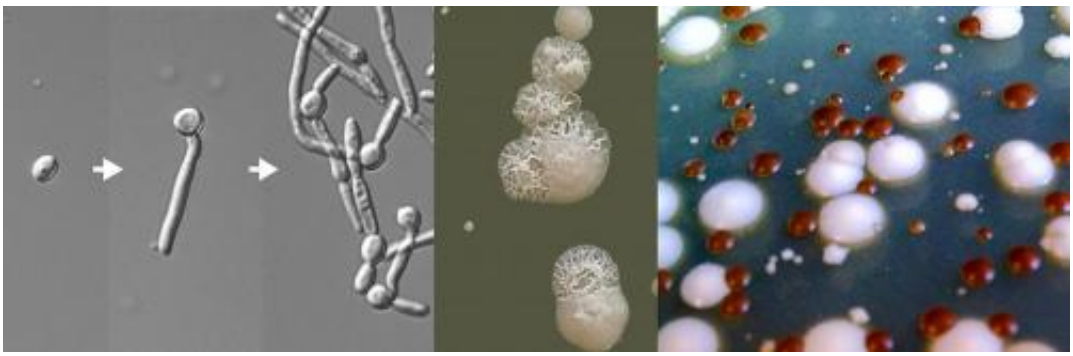

The fungal pathogen Candida albicans transitioning from the yeast form (round on the left) to a filamentous morphology. This transition is important for tissue invasion. Efficient mutagenesis of Ras1 (middle) and Ade2 (right) using CRISPR, which results in a change from a smooth to wrinkled colony morphology (which reflects a more filamentous state) or from white to red color, respectively. The high frequency of wrinkled and red reflects the high efficiency of mutagenesis with CRISPR, which is unmatched by previous technologies. Credit: Valmik K. Vyas and Tom DiCesare

By modifying the CRISPR-Cas genome editing system, Whitehead Institute researchers are now able to manipulate *Candida albicans*' genome systematically—an approach that could help identify novel targets for therapies against this serious pathogen for which there are a limited number of anti-fungal agents.

"The ability to engineer *Candida albicans* with CRISPR technology has changed the playing field," says Whitehead Founding Member Gerald Fink, who is also a professor of biology at MIT. "We used to attack this human pathogen with our hands tied behind our back. Our findings cut these bonds, freeing us to forge ahead on problems in basic research and human health."

C. albicans is a commensal organism that normally lives harmlessly on the skin or in the gut. However, this yeast can grow in uncontrolled fashion—particularly in immunocompromised individuals—causing fungal infections ranging from mild to lethal. *C. albicans* is a hardy foe because many strains are resistant to antifungal drugs. To develop new antifungal agents, researchers need to know more about its basic biology.

One tactic for identifying new drug targets in such pathogens is to knock out each of the organism's genes to determine which are essential and therefore appropriate as drug targets. The genome of *C. albicans* has been particularly difficult to crack because it has two copies of every gene and existing genome editing methods have been inefficient in knocking out both copies simultaneously.

In 2012, a bacterial immunity system —the clustered, regularly interspaced, short palindromic repeats (CRISPR)-associated protein 9 (Cas) system—was repurposed for genome editing. It is precise and efficient enough to edit both copies of a gene in most diploid organisms. However, *C. albicans*' unique genetic makeup renders the standard CRISPR-Cas system ineffective, requiring considerable modification. After extensive efforts, Valmik Vyas, a postdoctoral researcher in Fink's lab, engineered a CRISPR system that can work in *C. albicans* and most other fungi. Vyas describes his system is in this week's issue of the journal *Science Advances*.

Using his altered gene editing system in both laboratory and clinical

strains, Vyas efficiently mutated in a single experiment both copies of several different genes, including members of a gene family important for antibiotic resistance as well as an essential gene. Vyas estimates that his modified CRISPR-Cas system should be able to target more than 98% of *C. albicans*' genome. That means he should be able to determine which of *C. albicans*' 6000 genes are essential and might make good drug targets.

"The improvement efficiency brought by this system expands the scale at which we can do genetics in this important pathogen," says Vyas. "It's an exciting time to be working on Candida."

More information: "A Candida albicans CRISPR system permits genetic engineering of essential genes and gene families" *Science Advances*, April 3, 2015. advances.sciencemag.org/content/1/3/e1500248

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