**MINITOPIC**

Research Findings Tie Microbiota to Various Diseases

Researchers are continuing to investigate how the microbiota might be linked to a variety of diseases. Recent findings include:

- High levels of antibodies to *Porphyromonas gingivalis*, a pathogen from the oral cavity, correlate with a twofold elevated risk for pancreatic cancer, while high antibodies for commensal bacteria from that site are linked with a 45% lower risk, according to Dominique Michaud of Brown University in Providence, RI, and her collaborators. Details appear in the September 18, 2012 *Gut*.

- Microorganisms in the lungs of individuals with cystic fibrosis have a distinct “signature,” including reduced diversity, compared to those who do not have this disease, according to David Cornfield of Stanford University Medical School in Stanford, Calif., and his collaborators. Details appear in the September 26, 2012 *Science Translational Medicine*.

- A relatively high level of opportunistic pathogens, a decrease in butyrate-producing bacteria, and enrichments of microbial functions conferring sulfate reduction and oxidative stress resistance among bacteria of the gastrointestinal tract are linked to one’s risk of developing type 2 diabetes, according to Jun Wang and Kirsten Kristiansen from the University of Copenhagen in Copenhagen, Denmark, and their collaborators. Details appear in the September 26, 2012 *Nature* doi: 10.1038/nature11450.

- In driving inflammatory responses, the gut microbiota can promote the development of colorectal cancer in mice, according to Christian Jobin of the University of North Carolina at Chapel Hill and his collaborators. Details appear in the October 5, 2012 *Science* DOI: 10.1126/science.1224820.

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**RESEARCH ADVANCES**

Combating Drug-Resistant Fungi

Shannon Weiman

The molecular chaperone Hsp90 is what appears to be the Achilles’ heel of fungal pathogens—a target that, once inhibited, helps to avoid or overcome resistance to other antifungal drugs, according to Leah Cowen of the University of Toronto in Toronto, Ontario, Canada. However, because similar chaperone proteins are found in mammals, this approach will need some fine-tuning to avoid toxicities, she points out. Cowen spoke during the symposium, “New Approaches in the Treatment of Fungal Infections,” convened as part of the 2012 ICAAC, held in San Francisco last September.

Hsp90, which helps proteins to fold and function properly, including when subject to drug-induced stress, interacts with many fungal proteins. Among them, calcineurin, a calcium-activated phosphatase, is particularly critical, according to Cowen. “Hsp90 physically interacts with the catalytic subunit of calcineurin, maintaining it in stable conformation that is poised for activation,” she says. When activated, calcineurin dephosphorylates transcription factors such as Crz1, allowing them to translocate to the nucleus and activate gene expression programs that enhance fungal survival.

Inhibiting Hsp90 proves potent when combined with conventional antifungal drugs, she continues. “Azoles are fungistatic against *C. albicans* and inhibit growth but do not eradicate fungal burden, creating conditions that favor the emergence of drug resistance. In combination with Hsp90 inhibition, fungistatic azoles become fungicidal.”

Such Hsp90 inhibitors prevent fungi from developing resistance in vitro and, even more strikingly, restore drug susceptibility in drug-resistant clinical isolates. Hsp90 inhibitors are also effective in the context of biofilms, in which fungal pathogens are particularly resistant to treatment. In vivo, pharmacological inhibition or genetic manipulation of Hsp90 attenuates fungal virulence, rescuing animal hosts from lethal doses of drug-resistant fungi. These findings hold true for di-

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Micrograph of *Candida albicans* cells. *C. albicans* is among the fungal pathogens that are developing resistance to antifungal agents, complicating their treatment in human infections. Researchers have targeted the molecular chaperone Hsp90 as a means of preventing or overcoming resistance in fungal pathogens. (Photo © iStockphoto/Karl Dolenc.)
verse classes of fungi, including the yeast *Candida albicans* and mold *Aspergillus fumigatus*, in the face of several classes of antifungal drugs, including azoles and echinocandins.

Hsp90 inhibitors are well tolerated in humans and are currently in clinical trials as antineoplastic agents. However, in mice these drugs have detrimental effects in the context of infection by nonselectively inhibiting mammalian Hsp90, which impairs immune response. Cowen and her colleagues are in the process of screening for fungal-selective inhibitors, and have identified some leads that may be applicable in the clinical setting.

Alternatively, Cowen suggests targeting the downstream effector calcineurin. Calcineurin is regulated by KDACs, which deacetylate key lysine residues to enable functional conformation. Cowen has found that inhibiting KDACs is just as effective as inhibiting Hsp90 itself in both in vitro and in vivo studies examining antifungal drug resistance, biofilms, and host survival. KDAC inhibitors are approved for use in humans against leukemia and are in clinical trials for the treatment of other cancers; however, here too it will be important to identify fungal-selective agents. “The divergence of KDACs between fungi and humans is far greater than that for Hsp90, suggesting this might provide a more attractive strategy for antifungal therapy,” says Cowen.

“Ideally, a new therapeutic strategy for fungal infectious disease would enhance the efficacy of existing antifungal drugs, block the emergence of drug resistance, have fungicidal activity, and demonstrate broad efficacy against diverse fungal pathogens. We have established that harnessing fungal Hsp90 meets all of these criteria and has profound therapeutic benefits,” Cowen concludes.

**Shannon Weiman is a freelance writer in San Francisco, Calif.**

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**RESEARCH ADVANCES**

**Next-Generation Sequencing Provides Help in Growing “Uncultivable” Microbes**

**John Otrompke**

Faster methods of sequencing microbial genomes might hold a key to understanding those microbes well enough to culture some isolates that are considered among the most intracatable, according to Joerg Graf of the University of Connecticut in Storrs and his collaborators. He spoke during the 2012 International Symposium on Microbial Ecology (ISME), which convened last August in Copenhagen, Denmark.

“Sometimes these ‘unculturable’ microbes are in an inactive state, just hanging out in an environment, and some kind of signal has to revive them,” Graf says. “Other times, they require specific nutrients to allow them to proliferate.”

Graf applied this approach to the first-time culturing of an as-yet-unnamed microbe that is related to *Rikenella*, bacteria that are found in the digestive tracts of numerous animal species. “We had an organism we couldn’t cultivate, so we used 454 sequencing to see what the organism was like inside the host animal, and then could supply the nutrients it needed to grow,” he says, referring to a commercially available DNA sequencing procedure. The host for the *Rikenella*-like microbe is *Hirudo verbana*, a leech that is used in medicine, including to treat tissue grafts.

Metatranscriptomes reveal which genes in the microorganisms are highly expressed at a specific time, pointing to some of its critical nutritional preferences, according to Graf and his collaborators, some of whose earlier findings appear in the April 2011 *mBio* (doi: 10.1128/mBio.00012-11). During the 2012 ISME, he described how they are using 454 pyrosequencing to profile the microbial community within the leech gut. “Initially, we were thinking that the bacteria proliferated on something supplied in the leech’s blood meal,” he says. Instead, the *Rikenella*-like bacteria grow on host-provided mucin.

“Another reason this procedure can be challenging is because most RNA in bacterial cells is ribosomal RNA, which can be very challenging to remove,” Graf continues. “We could sequence 98% of it, but it does not really tell us what the physiology of the unculturable microorganism is like. But with this next-generation technique, we can just sequence through, because we can still sequence the messenger RNA, even though a lot of ribosomal RNA is left in the sample.”

“This research is significant in several ways,” says Angela Douglas of Cornell University in Ithaca, N.Y., who uses similar technologies to study microorganisms that associate with insects. “The 1980s saw a dramatic revolution in microbiology, when people showed you could identify a microorganism by its RNA without culturing it. We learned of a biodiversity we hadn’t imagined, and of two different classifications of microorganisms, the bacteria and archaea. It also led to the discovery of enormous viruses with very large genomes. It seems we’ve gone from the viewpoint that if we can’t culture it, we can’t study it, to the view where sequencing analysis almost replaces culturing, and now we’ve come full circle to where sequencing data can enhance our potential to culture an organism.”

**John Otrompke is a writer based in Chicago, Ill.**

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**RESEARCH ADVANCES**

**Laccase and Iodide Salts May Protect Wood against Molds**

**David C. Holzman**

Incubating wood with iodide salts and the oxidizing enzyme, laccase, yields a...