Aspartyl proteases govern *Candida glabrata*-epithelial cells interplay

Sandip Patra, Dr. Rupinder Kaur
Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India

Abstract

*Candida glabrata*, a non-spore-forming haploid budding yeast, resides as a commensal in the oral cavity and gastrointestinal and genitourinary tracts in healthy individuals. This opportunistic fungal pathogen causes mucosal and life-threatening systemic fungal infections upon immunocompromised conditions. Although over the last decade, antifungal drug resistance mechanisms and intracellular survival strategies of *C. glabrata* are increasingly being delineated, the interaction of *C. glabrata* with host epithelial and endothelial cells remains largely an unexplored area. In the current study, we have established an *in vitro* model system, using the human renal epithelial cell line A-498, and showed that *C. glabrata* intimately attaches to A-498 cells, and this epithelial cell-*C. glabrata* binding is blocked by sugar carbohydrates including lactose. These results indicate that the lectin family of cell wall adhesins may modulate host epithelial cell adherence. We further showed that similar to Lec2 cells, the family of eleven putative cell surface-associated aspartyl proteases (*CgYapsins*), encoded by *CgYPS1-11* genes, governs this process of epithelial cell adhesion. *CgYPS1-11* loss led to increased adherence and elevated cytokine production by A-498 cells. Using a combined approach of super resolution microscopy and colony-forming unit-based viability, immune response pathway activation, cytokine, mass spectrometry-based proteomic and CRISPR-Cas9 knock-out analyses, we have deciphered *Cg*-A-498 interplay in depth. These results, along with how the proteolytic activity of *CgYps1* and the interactors of *CgYps1* and *CgYps7* proteases, control adhesion to the host cells, will be presented.
Evolution of Acquired Resistance for Hydrogen Peroxide Involves Differential Sensing of Phosphate by TORC1 and Activation of the Transcriptional Factor Msn4 in *Candida glabrata*

Bin He, Jinye Liang
the University of Iowa, USA

Abstract

Environmental stresses often occur in combination or in close succession. Thus, the ability to anticipate upcoming stresses could provide an important fitness advantage. We found that *Candida glabrata*, an opportunistic yeast pathogen closely related to the baker’s yeast *S. cerevisiae* exhibits acquired stress resistance (ASR) to severe hydrogen peroxide (H$_2$O$_2$) stress after being phosphate starved. Similar treatments in *S. cerevisiae* resulted in a much weaker protection only after a prolonged primary stress exposure. Transcriptomic analysis revealed phosphate starvation induced a number of oxidative stress responsive genes only in *C. glabrata*, including the only catalase encoded by *CTA1*. *cta1Δ* not only rendered *C. glabrata* cells more sensitive to H$_2$O$_2$ but also abolished the phosphate starvation-induced ASR. Among known transcription factors (TFs) regulating *CTA1*, we found the general stress response TF Msn4 and the oxidative stress TF Skn7 jointly contributed to *CTA1* induction during phosphate starvation. Both Msn4 nuclear localization and *CTA1* induction upon phosphate starvation depended on the Target-of-Rapamycin Complex 1 (TORC1). Consistent with this, TORC1 was found to be rapidly inactivated upon phosphate starvation in *C. glabrata* but not *S. cerevisiae*. *CTA1* induction during phosphate starvation was also diminished by genetically activating PKA or deleting Rim15, a kinase negatively regulated by both PKA and TORC1. Together, our results demonstrated that organisms' ability to predict upcoming stress is an evolvable trait that involves modifying the activity of conserved nutrient and stress-sensing regulators such as TORC1 and Msn4.
Parallel Expansion and Divergence of an Adhesin Family in Pathogenic Yeasts

Bin He, Rachel Smoak, Lindsey Snyder, Jan Fassler

the University of Iowa, USA

Abstract

Opportunistic yeast pathogens evolved multiple times in the Saccharomycetes class, including the recently emerged, multidrug-resistant Candida auris. We show that homologs of a known yeast adhesin family in Candida albicans, the Hyr/Iff-like (Hil) family, are enriched in distinct clades of Candida species as a result of multiple, independent expansions. Following gene duplication, the tandem repeat-rich region in these proteins diverged extremely rapidly and generated large variations in length and β-aggregation potential, both of which were known to directly affect adhesion. The conserved N-terminal effector domain was predicted to adopt a β-helical fold followed by an α-crystallin domain, making it structurally similar to a group of unrelated bacterial adhesins. Evolutionary analyses of the effector domain in C. auris revealed relaxed selective constraint combined with signatures of positive selection, suggesting functional diversification after gene duplication. Lastly, we found the Hil family genes to be enriched at chromosomal ends, which likely contributed to their expansion via ectopic recombination and break-induced replication. Combined, these results suggest that the expansion and diversification of adhesin families generate variation in adhesion and virulence within and between species and are a key step toward the emergence of fungal pathogens.
Yak1 Family Kinases Regulate *Candida albicans* Morphogenesis and Virulence through the Ras1/PKA Pathway

Jessie MacAlpine, Zhongle Liu, Luke Whitesell, Nicole Robbins, Leah Cowen

Department of Molecular Genetics, University of Toronto, Canada

Abstract

*Candida albicans* is an opportunistic human fungal pathogen that causes common mycoses in healthy populations and invasive disease in immunocompromised individuals. The fungus employs several virulence traits to cause disease in humans, including its ability to transition from yeast to filamentous morphologies. Previous work identified that genetic or pharmacological inhibition of the dual-specificity tyrosine-phosphorylation regulated kinase (DYRK) Yak1 blocks *C. albicans* hyphal morphogenesis and biofilm formation. Here, we expand on this work to provide mechanistic insights into how Yak1 governs this important virulence trait. First, we find that Yak1 acts downstream of PKA and upstream of core transcription factors to regulate filamentation in response to diverse inducing cues. We also report that hyperactivation of the Ras1/cAMP/PKA pathway under physiological concentrations of CO$_2$ bypasses the requirement for Yak1 in the yeast-to-filament transition. In an attempt to identify the factor(s) downstream of PKA that mediates hyphal morphogenesis under elevated CO$_2$, we describe a novel role for the related kinase, Pom1, in regulating filamentation, as a homozygous deletion of both *yak1* and *pom1* blocked filamentation even under elevated CO$_2$. Finally, we demonstrate that Yak1 is required for hyphal morphogenesis in a dermatitis model of *C. albicans* infection and that pharmacological inhibition of Yak1 with a beta-carboline attenuates filamentation in the dermal tissue. Overall, this work characterizes the role of Yak1 in regulating *C. albicans* morphogenesis, identifies an undescribed role for Pom1 in the yeast-to-filament transition, and suggests inhibition of Yak1 may serve as a therapeutic strategy to combat *C. albicans* dermatitis.
Investigating the Role of the R Chromosome in Drug Resistance in Candida albicans via CRISPRa Screening

Nicholas Gervais¹, Meea Fogal¹, Lauren Wensing¹, Abdul-Rahman Adamu Bukari², Aleeza Gerstein², Rebecca Shapiro¹

¹University of Guelph, Canada. ²University of Manitoba, Canada

Abstract

The emergence of drug-resistant Candida strains is a critical threat to human health globally. One mechanism of acquiring drug resistance that Candida albicans harnesses is its ability to readily undergo chromosomal rearrangements and alter ploidy to overcome stressful conditions such as antifungal drug treatment. However, the genetic pathways underpinning many aneuploidies are uncharacterized. It has recently been shown that a trisomy of chromosome R (ChrR) in C. albicans results in increased resistance and tolerance to certain antifungal drugs. This trisomy suggests that overexpression of one or more genes on ChrR is associated with antifungal drug response phenotypes. Better characterization of ChrR and its corresponding genes through genetic manipulation will therefore promote our understanding of the molecular mechanisms mediating the evolution of resistance to antifungal drugs. Here, we propose the development of a novel CRISPR-based overexpression library that will target ChrR in C. albicans and will allow genes and genetic mechanisms relating to antifungal drug resistance to be discovered. We have recently optimized a new CRISPRa (activation) system in C. albicans and have employed it in single-gene perturbation studies. Scaling this technology up to target an entire chromosome allows us to overexpress one gene on ChrR per mutant strain, thus mimicking the gene dosage conditions present when there is an extra copy of ChrR present in drug-resistant C. albicans strains. Our resulting data will therefore help to uncover fundamental mechanisms that influence antifungal drug response as well as establish an important CRISPR screening platform in C. albicans.
BDNG001 dendrimer: a compound capable of eradicating a mixed biofilm

Natalia Gómez-Casanova¹, Cristina Verdú-Expósito¹, Javier Sánchez-Nieves², José Luis Copa- Patoiño¹, Irene Heredero-Bermejo¹

¹University of Alcalá, Department of Biomedicine and Biotechnology, Faculty of Pharmacy., Spain.
²University of Alcalá, Department of Organic and Inorganic Chemistry, Research Institute in Chemistry "Andrés M. del Río" (IQAR), Madrid, Spain. Institute "Ramón y Cajal" for Health Research (IRYCIS), Spain. Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain

Abstract

Biofilms are generally considered as a main health issue, especially in immunocompromised patients. These communities of microorganisms are difficult to eliminate, and treatment is more complicated for those developed by more than one type of microorganism, called mixed biofilms, such as those formed by fungi and bacteria. Two of the most common clinical isolates are related to human infections and biofilms infections are Candida albicans and Staphylococcus aureus. These pathogens can easily develop biofilms on different medical devices, such as catheters, implants, or dialysis units. The problematic associated to these biofilms is that antimicrobials and antifungals with activity to eradicate them are becoming more and more limited. Therefore, the development of mixed biofilms is a significant and difficult health problem to address. Mixed biofilm between yeast (Candida albicans ATCC18804) and bacteria (Staphylococcus aureus ATCC6538P) was optimized using crystal violet. Then, a new dendritic cationic compound, BDNG001 dendrimer, was tested against mixed biofilm formation and against established mixed biofilms. The viability of these strains was assessed by a resazurin colorimetric assay and confirmed on agar plates. Finally, cytotoxicity was tested on HeLa cell line. We verified a homogeneity in the biomass of the mixed biofilms. In mixed biofilm formation studies, BDNG001 dendrimer showed a Minimum Biofilm Inhibitory Concentration (MBIC) of 4 mg/L; and in established mixed biofilm studies, BDNG001 dendrimer showed a Minimum Biofilm Eradication Concentration (MBEC) of 32 mg/L. BDNG001 was non-cytotoxic for this actives concentration. These results are very promising and encourage further studies related to this compound.
New dendritic cationic compound capable of preventing the formation of biofilm in *Candida* spp.

Cristina Verdú-Expósito¹, Natalia Gómez-Casanova¹, Javier Sánchez-Nieves², José Luis Copa-Patiño¹, Irene Heredero-Bermejo¹

¹University of Alcalá, Department of Biomedicine and Biotechnology, Faculty of Pharmacy, Madrid, Spain. ²University of Alcalá, Department of Organic and Inorganic Chemistry, Research Institute in Chemistry "Andrés M. del Río" (IQAR), Madrid, Spain. Institute "Ramón y Cajal" for Health Research (IRYCIS), Spain. Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain

Abstract

Infections caused by pathogenic *Candida* are widely known. In particular, the species *Candida auris* is one the most concerning. Their resistance to antifungal is gradually increasing, and it is more difficult to find antifungals capable of controlling these pathogens, especially in the biofilm form. Once a stable biofilm has developed, it is very difficult to break up and eliminate the cells that form this structure. Normally, high concentrations are required to remove them and, consequently, these concentrations are very toxic. Therefore, preventing these strains from producing biofilms is crucial, especially in debilitated and hospitalized patients such as neonates, immunosuppressed, and patients with catheters, among others. *In vitro* activity of a new dendritic cationic compound, BDNG001 dendrimer, was tested on *C. albicans* ATCC 18804, *C. glabrata* ATCC 2001, *C. auris* ATCC 5001 and a clinical *C. auris* strain to evaluate its effect on biofilm formation. In the biofilm assay, the viability of this strains was assessed by a resazurin colorimetric assay and confirmed on agar plates. Then, cytotoxicity was tested on HeLa cell line. BDNG001 dendrimer showed a Minimum Biofilm Inhibitory Concentration (MBIC) of 8 mg/L against all *Candida* strains, including the clinical *C. auris*. BDNG001 dendrimer was non-cytotoxic at the effective concentration. We conclude that the BDNG001 dendrimer has a high activity against *Candida* spp, even against those species that are a current public health problem. Future studies will allow us to better understand the scope of the activity of this compound.
Discovery of Novel Triazoles Containing Benzyloxy Phenyl Isoxazole Side Chain with Potent and Broad-Spectrum Antifungal Activity

Tingjunhong Ni¹, Dazhi Zhang², Lan Yan², Yuanying Jiang¹

¹Department of Pharmacy, Shanghai Tenth People's Hospital, School of Medicine, Tongji University, China. ²School of Pharmacy, Naval Medical University, China

Abstract

As a continuation study, 29 novel triazoles containing benzyloxy phenyl isoxazole side chain were designed and synthesized based on our previous work. All compounds exhibited moderate to excellent in vitro antifungal activities against eight pathogenic fungi. The most active compounds 13, 20 and 27 displayed outstanding antifungal activity with MIC values ranging from < 0.008 µg/mL to 1 µg/mL, and showed potent activity against six drug-resistant Candida auris isolates. Growth curve assays further confirmed the high potency of these compounds. Moreover, compounds 13, 20 and 27 showed a potent inhibitory activity on biofilm formation of C. albicans SC5314 and C. neoformans H99. Notably, compound 13 showed no inhibition of human CYP1A2 and low inhibitory activity against CYP2D6 and CYP3A4, suggesting a low risk of drug-drug interactions. With high potency in vitro and in vivo and good safety profiles, compound 13 will be further investigated as a promising candidate.
Interfering fungal membrane sterol homeostasis with the accumulation of eburicol and/or obtusifoliol helps azoles produce fungicidal effect

Jiacun Liu¹, Ruina Wang¹, Quanzhen Lv¹, Tingjunhong Ni², Yuanying Jiang², Dazhi Zhang¹, Lan Yan¹

¹School of Pharmacy, Naval Medical University, China. ²Department of Pharmacy, Shanghai Tenth People's Hospital, School of Medicine, Tongji University, China

Abstract

Fungistatic azoles have been the most widely used treatment for systemic candidiasis for decades. Combination therapies with agents have been a promising strategy to increase the lethality of azoles. In this study, several chemical compounds were designed and synthesized based on berberine derivatives. In vitro evaluations showed that most compounds had synergistic antifungal activity against the fluconazole-resistance Candida albicans with azoles. In particularly, the combination of G42 and azoles had fungicidal effect against both fluconazole sensitive and resistant strains. In comparison with the treatment of fluconazole, the combination of G42 and fluconazole significantly increased the expression of ERG6, and abnormally accumulated eburicol and obtusifoliol in the membrane sterol composition. The deletion of ERG6 led to slow growth rate owing to its major sterol of zymosterol. It showed that in the erg6 null mutant, although G42 enhanced the inhibition of fluconazole on the target ERG11 and produced a large amount of lanosterol accumulation, it did not further produce toxic eburicol or obtusifoliol because of the loss of ERG6, which resulted in resistance to fluconazole or even the combination of fluconazole and G42. Moreover, the deletion of ERG3 was resistant to fluconazole but sensitive to the addition of G42, resulted from the accumulation of eburicol. Therefore, the accumulation of eburicol and/or obtusifoliol caused by the inhibition of ERG25/26/27 is lethal in fungi, which is an ideal strategy to overcome theazole tolerance. The leading compounds such as G42 have paved the way to obtain potential synergistic fungicidal agent with azoles.
Engineering drug sensitivity to screen for new classes of antibiotics for the emerging, drug resistant fungal pathogen Candida auris.

Mahnoor Hassan¹, A. James Mason¹, J. Mark Sutton², Charlotte Hind², Barry Panaretou¹

¹King’s College London, United Kingdom. ²UK Health Security Agency, United Kingdom

Abstract

Candida auris is an emerging, multi-drug resistant, pathogenic fungus responsible for an increase in invasive fungal infections worldwide. C. auris infections are concerning for three particular reasons: i) it has been misidentified for numerous years; ii) it persists in hospital environments, posing an increased risk for immunocompromised individuals and; iii) it exhibits an increased frequency of drug-resistance, with numerous drug transporter genes up-regulated in drug-resistant C. auris isolates. Therefore, a need for new antifungal treatments has become much more imminent.

Here we show that deletion of CDR1 and MDR1 in C. auris, respectively encoding an ATP-binding Cassette and a Major Facilitator Superfamily drug pump, generates a strain that could act as a platform for antifungal drug screening. Targeted homologous recombination of a nourseothricin resistance cassette is used to disrupt CDR1 followed by excision of the drug resistance marker via cre-lox mediated recombination, to enable its re-use to delete MDR1.

The cdrΔDmdr1Δ mutant has respectively a 64-fold and 8-fold increase in sensitivity to fluconazole and voriconazole, compared with the wild-type strain. However, no significant difference between wild-type and double mutant is observed for biofilm formation, nor virulence as judged by a Galleria mellonella infectivity assay. Accordingly, we can now exploit these strains to investigate the effects of drugs on both of these key properties associated with C. auris without being concerned about effects caused by deletion of the drug pump genes.
Candida auris controls surface colonization through the unique adhesin SCF1.

Darian Santana, Teresa O'Meara
University of Michigan, USA

Abstract

Candida auris is an emerging fungal pathogen responsible for healthcare-associated outbreaks driven by persistent surface colonization. We functionally characterized the arsenal of adhesins utilized by C. auris for surface attachment and discovered that Surface Colonization Factor (SCF1), an adhesin unique to C. auris, and IFF4109, a conserved IFF/HYR family adhesin, are major drivers of attachment and are critical for biofilm formation. Transcriptional control of SCF1 varied widely among C. auris isolates and was positively associated with adhesion across all five clades. Unlike canonical adhesins which mediate surface attachment via hydrophobic interactions, SCF1 relies on cation-substrate interactions in a manner analogous to barnacle and mussel adhesion proteins. These findings explain the ability of C. auris to colonize abiotic surfaces, a trait critical for its pathogenesis.
Diverse environmental signals mediate changes in cell wall PAMPs at the *Candida albicans* cell surface thereby contributing to immune evasion during systemic infection.

Arnab Pradhan¹, Qinxi Ma¹, Emer Hickey¹, Gabriela Avelar², Daniel Larcombe¹, Judith Bain², Delma Childers², Ivy Dambuza¹, Ian Leaves¹, Leandro Jose de Assis¹, Mihai Netea³,⁴, Gordon Brown¹, Lars Erwig²,⁵, Neil A.R. Gow¹, Alistair J.P. Brown¹

¹MRC Centre for Medical Mycology, University of Exeter, Exeter, United Kingdom. ²Institute of Medical Science, University of Aberdeen, Aberdeen, United Kingdom. ³Department of Internal Medicine and Radboud Centre for Infectious Diseases, Radboud University Medical Centre, Nijmegen, Netherlands. ⁴Department for Immunology & Metabolism, Life and Medical Sciences Institute (LIMES), University of Bonn, 53115 Bonn, Germany. ⁵Johnson-Johnson Innovation, EMEA Innovation Centre, One Chapel Place, London W1G 0BG, United Kingdom

Abstract

The cell wall has a major impact upon host-fungus interactions as it is the first point of direct contact with the host. *Candida albicans* has evolved immune evasion strategies that include the remodelling of its cell wall and associated pathogen-associated molecular patterns (PAMPs). We performed a cytometric screen for host inputs that influence the exposure of β-(1,3)-glucan, a major proinflammatory PAMP, at the *C. albicans* cell surface. Inputs such as nitrogen sources and quorum sensing molecules exhibited limited effects on β-glucan exposure, whereas micronutrient limitation, certain stresses and antifungal drugs triggered significant changes in β-glucan exposure. Certain conditions were then analyzed in more detail using time-lapse video microscopy of *C. albicans*-phagocyte interactions, cytokine assays, flow cytometry, and fluorescence and transmission electron microscopy. Exposure to lactate, hypoxia or iron limitation induced β-glucan masking and attenuated innate immune responses. Each of these inputs was found to trigger β-glucan masking via different upstream signaling pathways, but all depended upon downstream signaling via the cAMP-PKA pathway. We then tested whether preadaptation to β-glucan masking signals affects fungal fitness during the early stages of a systemic infection. Differentially adapted cells were compared directly in a murine model using barcode-sequencing, revealing that β-glucan masking signals promote the colonisation of particular tissues. We conclude that *C. albicans* exploits certain specific host-derived signals to limit β-glucan exposure at its cell surface in an attempt to evade antifungal immunity during infection.
A conserved polyprotein structure escorts the peptide toxin candidalysin through the secretory pathway in *Candida albicans*

Rita Müller¹, Annika König², Tom Handrianz², Sabrina Groth³, Robert Zarnowski³, Corissa Visser⁴, Corinne Maufrais⁵, Sejeong Lee⁶, Emily L. Priest⁶, Deniz Yıldırım¹, Jonathan P. Richardson⁶, Matthew G. Blango⁷, Marie-Elisabeth Bougnoux⁸, Christophe d’Enfert⁶, Axel A. Brakhage⁴, David R. Andes³, Christian Nehls², Lydia Kasper¹, Selene Mogavero¹, Thomas Gutsmann², Julian R. Naglik⁶, Stefanie Allert¹, Bernhard Hube¹,¹⁰

¹. Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute (HKI), Germany. ². Division of Biophysics, Research Center Borstel, Leibniz Lung Center, Germany. ³. Department of Medicine, Section of Infectious Diseases, University of Wisconsin-Madison, USA. ⁴. Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute (HKI), Germany. ⁵. Institute Pasteur, Université Paris Cité, Bioinformatics and Biostatistics Hub, France. ⁶. Centre for Host-Microbiome Interactions, Faculty of Dentistry, Oral & Craniofacial Sciences, King’s College London, United Kingdom. ⁷. RNA Biology of Fungal Infections, Leibniz Institute for Natural Product Research and Infection Biology-Hans Knoell Institute (HKI), Germany. ⁸. Institute Pasteur, Université Paris Cité, Unité Biologie et Pathogénicité Fongiques, France. ⁹. Unité de Parasitologie-Mycologie, Service de Microbiologie Clinique, Hôpital Necker-Enfants-Malades, Assistance Publique des Hôpitaux de Paris (APHP), France. ¹⁰. Institute of Microbiology, Friedrich Schiller University, Germany

Abstract

*Candida albicans* damages host cells via its peptide toxin, candidalysin. Prior to secretion, candidalysin is embedded in a precursor protein, Ece1, consisting of a signal peptide, the precursor of candidalysin, and seven non-candidalysin Ece1 peptides (NCEPs). Here, we show that the exceptional Ece1 structure does not conform to the canonical definition of a toxin-antitoxin system or resemble the usual precursor structure of peptide toxins. *C. albicans* cells are protected from damage by their own toxin, and single NCEPs adjacent to candidalysin are sufficient to prevent host cell toxicity. Critically, we show that NCEPs play a vital role for intracellular Ece1 folding and candidalysin secretion. Removal of single NCEPs or modifications of peptide sequences cause an unfolded protein response (UPR), which in turn inhibits hypha formation and pathogenicity. Our data indicate that the Ece1 precursor is not required to block premature pore-forming toxicity, but rather to prevent candidalysin auto-aggregation.
Genetic modification of Candida maltosa, a nonpathogenic CTG species

Marco Antonio Chávez Tinoco, Luis F García Ortega, Eugenio Mancera

Centro de Investigación y de Estudios Avanzados del IPN unidad Irapuato, Mexico

Abstract

Candida maltosa is closely related to important pathogenic Candida species, especially C. tropicalis, but to our knowledge it has never been isolated from humans. For this reason, it is an ideal organism to understand the genetic underpinnings of the pathogenicity of Candida species through comparative studies. The aim of this research was to generate a better assembly of the C. maltosa genome and to develop genetic engineering tools that will allow us to study this species at a molecular level. To this end, we used short and log-read sequencing to build a polished genomic assembly composed of 14 Mbp, 58 contigs and 5,518 genes. Using the Saccharomycetes BUSCO database, genome completeness was estimated to be close to 96%. This assembly represents a substantial improvement from the currently available sequence that is composed of thousands of contigs. To be able to edit the genome of C. maltosa we generated a set of triple auxotrophic strains so that gene deletions can be performed similarly to what has been routinely done in pathogenic Candida species. We used the FLIP-SAT system optimized for C. tropicalis to tandemly delete LEU2, HIS1 and ARG4. The triple auxotrophic strains allow deletion of the two alleles of a given gene and its subsequent reintegration for complementation assays. As a proof of concept, we generated knockouts of genes associated to biofilm formation in other Candida species. The auxotrophic mutants and the new genome assembly are a key step to start using C. maltosa for comparative and evolutionary studies.
Funding Opportunities at the National Institute of Dental & Craniofacial Research (NIDCR)

Amanda Melillo

National Institute of Dental and Craniofacial Research, NIH, USA

Abstract

The National Institute of Dental and Craniofacial Research (NIDCR) is one of the 27 institutes that make up the US National Institutes of Health (NIH). The mission of NIDCR is to improve oral, dental, and craniofacial health through research, research training, and the dissemination of health information. Through the Oral Opportunistic Pathogens and Viral Disease Program, NIDCR supports extramural basic and translational research on the mechanism of pathogenesis of Candida species including inter-kingdom microbial interactions. NIDCR encourages research on characterizing host factors and the underlying immune, molecular and cellular mechanisms for the elimination of opportunistic pathogens in oral reservoirs.
Imaging-based screening identifies eIF3 complex as a new anti-virulence target in *C. albicans*

Katie Metzner, Matthew J O'Meara, Benjamin Halligan, Jonathan Z Sexton, Teresa R O'Meara

University of Michigan, USA

Abstract

*Candida albicans* is a fungal pathogen which can cause devastating human disease, especially in immunocompromised individuals. With minimal options for antifungal drugs, coupled with high levels of resistance and host toxicity, candidemia infections can be notoriously difficult to treat. This dilemma demonstrates the need for novel antifungal therapies. Promising targets for these new therapies are virulence factors. An important virulence factor in *C. albicans* is the ability to transition between two morphological states: yeast and hyphae. Using a high throughput automated microscopy and image analysis pipeline which can distinguish between yeast and hyphae, we screened an FDA drug repurposing library for compounds which inhibit the transition from yeast to hyphae in a dose-responsive manner. We identified 13 novel compounds which displayed efficacy and dose-responsive behavior. Three of these compounds shared a similar chemotype, and we therefore focused on these to determine mechanism of action. We evolved resistance to the three compounds and used whole-genome sequencing and allele swap experiments to identify the translation initiation complex eIF3 as a novel regulator of filamentation. Future work will include analysis of additional compounds, including a subset of the DART library of diverse drug-like small molecules, to identify additional pathways for limiting *C. albicans* virulence factors.
Human commensal *Candida albicans* strains demonstrate substantial within-host diversity and retained pathogenic potential

Faith Anderson, Noelle Visser, Kevin Amses, Andrea Hodgins-Davis, Alexandra Webb, Katura Metzner, Michael McFadden, Ryan Mills, Matthew O'Meara, Timothy James, Teresa O'Meara

University of Michigan, USA

Abstract

*Candida albicans* is a frequent colonizer of human mucosal surfaces as well as an opportunistic pathogen. *C. albicans* is remarkably versatile in its ability to colonize diverse host sites with differences in oxygen and nutrient availability, pH, immune responses, and resident microbes, among other cues. It is unclear how the genetic background of a commensal colonizing population can influence the shift to pathogenicity. Therefore, we undertook an examination of commensal isolates from healthy donors with a goal of identifying host niche-specific adaptations. We demonstrate that healthy people are reservoirs for genotypically and phenotypically diverse *C. albicans* strains. Using limited diversity exploitation, we identified a single nucleotide change in the uncharacterized ZMS1 transcription factor that was sufficient to drive hyper invasion into agar. We found that SC5314 was an outlier in its ability to induce host cell death compared to both commensal and bloodstream isolates. However, our commensal strains retained the capacity to cause disease in the Galleria model of systemic infection, including outcompeting the SC5314 reference strain during systemic competition assays. This study provides a global view of commensal strain variation and within-host strain diversity of *C. albicans* and suggests that selection for commensalism in humans does not result in a fitness cost for invasive disease.
Redefining Pleiotropic Drug Resistance in *Candida glabrata*: Pdr1 Senses Cellular Stresses, not Xenobiotics

Andrew Gale¹, Matthew Pavesic¹, Tim Nickels¹, Zhuwei Xu², Brendan Cormack², Kyle Cunningham¹

¹Department of Biology, Johns Hopkins University, USA. ²Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, USA

Abstract

*Candida glabrata* utilizes the transcription factor Pdr1 to up-regulate expression of several ABC transporters that together promote pleiotropic drug resistance. Spontaneous gain-of-function mutations in Pdr1 have been linked to fluconazole resistance and clinical failure. Earlier studies have proposed that Pdr1 directly senses fluconazole, cycloheximide, and other xenobiotics through direct binding to its core domain. We present new evidence that argues against this simple hypothesis and suggests instead that Pdr1 indirectly senses fluconazole, cycloheximide, and oligomycin by responding to the cellular stresses caused by the engagement of xenobiotics with their targets. Three types of evidence support this conclusion. First, hypomorphic mutations in targets of fluconazole (*ERG11*), cycloheximide (*RPL9* and *RPL28*), and oligomycin (*ATP1*) express *CDR1* at high basal levels, thus phenocopying the effects of the xenobiotics and triggering resistance to fluconazole. Second, affinity mutations in *ERG11* and *RPL28* that diminish the affinity for fluconazole and cycloheximide also diminished the potency of these compounds on Pdr1 activation and *CDR1* expression. These findings suggest fluconazole and cycloheximide must engage and inhibit their targets in order to activate Pdr1. Third, fluconazole, cycloheximide, and oligomycin activated Pdr1 and induced *CDR1* expression with very different kinetics. The delayed kinetics of Pdr1 activation by fluconazole correlated with delayed onset of growth defects, which first appeared after 90 minutes of exposure. Thus, Pdr1 appears to sense cellular stresses caused by xenobiotics rather than the xenobiotics themselves. We suspect that Pdr1 senses metabolites that increase or decrease in concentration following xenobiotic inhibition of translating ribosomes, mitochondria, and ergosterol biosynthesis.
C020

Identifying and characterizing genes important for *Candida albicans* fitness in diverse environmental conditions

Emily Xiong\(^1\), Xiang Zhang\(^2\), Huijuan Yan\(^3\), Ci Fu\(^1\), Nicole Robbins\(^1\), Suzanne Noble\(^3\), Chad Myers\(^2\), Leah Cowen\(^1\)

\(^1\)University of Toronto, Canada. \(^2\)University of Minnesota, USA. \(^3\)University of California San Francisco, USA

Abstract

*Candida albicans* is a leading cause of fungal infections in humans. Its impact on global human health is devastating, with mortality rates as high as 40% despite treatment. As the limited arsenal of effective antifungals in clinical use is threatened by emerging resistance, the demand for new therapeutic strategies to combat invasive fungi is urgent. A promising strategy to expand the therapeutic target space is to identify genes important for pathogen growth in environments relevant to the human host. This project aims to identify and characterize genes important for *C. albicans* fitness within host-relevant environments. This will be accomplished by leveraging a collection of barcoded *C. albicans* conditional-expression mutants. Pooled screening of this large-scale mutant library in minimal nutrient medium at 30 °C identified a novel essential gene with no known *Saccharomyces cerevisiae* homolog, *C1_09670C*. Follow-up computational, genetic, and phenotypic analyses predicted this open reading frame (ORF) to encode subunit 3 of replication factor A (RFA). Further screens performed in additional conditions, including at physiological temperature, low iron, and serum-supplemented medium, generated a rich dataset of genes important for fitness under specific conditions. This approach identified *C3_06880W*, an uncharacterized gene required for fitness in physiological temperatures with no functional predictions to date. Future work will explore mechanisms through which *C3_06880W*, and additional genes with condition-specific growth phenotypes, regulate *C. albicans* fitness. This work will reveal novel insights into vulnerabilities of *C. albicans*, which could be exploited to enable the development of new therapeutic strategies.
Developing novel microbial therapeutics to prevent systemic *Candida albicans* infections arising from the gastrointestinal tract

Bianca Briscas, Harry Tiernan, Matt Prokop, Megan Lenardon

UNSW Sydney, Australia

Abstract

Over 60% of humans have *Candida albicans* in their gastrointestinal tract and this represents a major risk factor for developing fatal invasive candidiasis. In healthy individuals, *C. albicans* is kept in check by competition with the gut bacterial microbiota, intact intestinal epithelial barriers, and by the host’s immune system. However, if the status of these host defences changes, then *C. albicans* can escape the gut, disseminate through the blood and cause systemic disease.

Expanding on a pilot study which utilised faecal samples from six healthy adult donors and a novel *in vitro* colon model to identify three bacterial species that kill *C. albicans* (Ricci et al. 2022 *FEMS Microbiol*), we obtained 27 new faecal samples from healthy adult donors. 16S rRNA gene sequencing, ITS1 sequencing and multivariate statistical analyses enabled the identification of several new bacterial and fungal taxa that are associated with the killing of *C. albicans*. We are testing whether the application of these potentially therapeutic bacterial and fungal species can clear *C. albicans* from our *in vitro* colon model, as well as an *ex vivo* gut organoid model.

The overall aim of this work is to demonstrate the feasibility of a non-invasive, targeted microbial therapeutic which clears *C. albicans* from the GI tract to prevent life-threatening disease. This represents a new way of thinking with respect to the management of invasive candidiasis, providing an alternative to current clinical reliance on antifungal drugs which do not always prevent death, and against which resistance is emerging.
Candida albicans from catheterized patients with persistent candidemia show enhanced biofilm growth in vitro, and dispersed cells from these biofilms are virulent entities.

Felipe de camargo Ribeiro¹, Arnaldo Lopes Colombo¹, Priya Uppuluri²

¹Laboratório Especial de Micologia, Disciplina de Infectologia, Universidade Federal de São Paulo, São Paulo, Brazil. ²The Lundquist Institute, David Geffen School of Medicine at UCLA, Division of Infectious Diseases, Harbor-UCLA Medical Center, USA

Abstract

Background: We have previously shown that cells dispersed from C. albicans biofilms are significantly more virulent in mice than their planktonic counterparts. We questioned, do these properties also translate in the clinic? Methods: C. albicans isolates representative of two groups were used for the study: Group I (G1), 5 isolates from the first day of infection of patients with persistent candidemia (baseline cultures); and Group II (G2), isolates from the same patients after several days of persistent candidemia despite 72h of antifungal therapy. All patients harbored central venous catheters. Dispersed cells were obtained from biofilms developed under the flow system, and characterized for their virulence properties: adherence and biofilm formation (XTT assay), tissue damage (51Cr release from HEK 293 cell line) and immune evasion (opsonophagocytosis by the RAW 264.7 cells). All the assays were also compared to the laboratory strain SC5314. Results: Persistent bloodstream isolates of C. albicans (G2) developed significantly better biofilms compared to their patient-matched isolates (G1) or SC5314. In fact, cells dispersed from G2 biofilms displayed higher capacity of adherence (p = 0.0119), higher biofilm production (p = 0.0158) and higher immune evasion (p = 0.007), compared to G1 or SC5314. Finally, isolates from G2 also displayed an increase in cell damage. Conclusion: We demonstrated that, C. albicans from prolonged candidemia can attach robustly to indwelling medical devices, and dispersed cells from these biofilms have enhanced properties of virulence.
Investigating the role of the *Candida albicans* telomeric TLO gene family in Tac1-mediated fluconazole resistance

Wen Jun Lim, Derek Sullivan, Gary Moran
Trinity College Dublin, Ireland

Abstract

**Background**

Gain-of-function (GOF) mutations in the transcriptional activator Tac1 result in the acquisition of azole resistance in *C. albicans* due to overexpression of genes encoding efflux pumps. This regulatory interaction is facilitated by Mediator complex. In *C. albicans* the Mediator tail subunit homologous to Med2 is encoded by a massively expanded gene family, the TLO (telomere associated ORF) genes. Here we determine if deletion of TLO genes in *C. albicans* affects Tac1-mediated fluconazole resistance.

**Methods**

CRISPR-Cas9 mutagenesis was used to introduce the hyperactive TAC1-5<sup>GOF</sup> mutation (N977D) into *C. albicans* wild type (MAY1244), tloΔ and med3Δ mutants. TLO genes representing different clades; TLO1 (clade α), TLO2 (clade β), and TLO11 (clade γ), were reintegrated into the tloΔ + TAC1-5 GOF mutant. qPCR was used to measure the gene expression of CDR1 and CDR2. Spot assays, disk diffusion tests and antifungal strips (E-test) were used to test antifungal susceptibility.

**Results**

Both tloΔ and med3Δ strains have lower CDR1 gene expression compared to the wild type. Hyperactive TAC1-5<sup>GOF</sup> alleles increased the fluconazole resistance of WT strains but only partially increased the fluconazole resistance of tloΔ and med3Δ strains. The reintegration of TLOα1 and TLOβ2 but not TLOγ11 restored TAC1-5 mediated fluconazole resistance of tloΔ. The tloΔ strain was found to be hypersensitive to terbinafine. Unexpectedly, while TLOα1 restored terbinafine resistance, the reintegration of TLOβ2 reversed TAC1-5<sup>GOF</sup> mediated terbinafine resistance.

**Conclusion**
Deletion of TLO and Med3 genes in *C. albicans* reduced Tac1-mediated fluconazole and terbinafine resistance implicating TLO gene as a potential drug target.
Selection of ethanol tolerant strains of Candida albicans by repeated ethanol exposure results in strains with cross-tolerance to fluconazole

Andrew Day, Carol Kumamoto
Tufts University, USA

Abstract

Alcohol use and abuse results in 3 million deaths each year and many factors influence recovery of individuals with alcohol use disorder (AUD). Recently, it has been shown that Candida albicans (Ca) blooms in the alcoholic GI tract and contributes to the development and progression of alcoholic liver disease. To understand how Ca responds to ethanol exposure, we initiated a study of the response of Ca to repeated ethanol exposure, similar to conditions in the upper GI tract of individuals with AUD. Here we show that populations of Ca repeatedly exposed to ethanol yield small colony variants (SCVs) that showed increased ethanol tolerance. One SCV was analyzed in detail and exhibited increased production of ergosterol and altered expression of ergosterol metabolism genes, including ERG11. As Erg11 is the target of the antifungal drug, fluconazole, we examined fluconazole susceptibility of the SCVs and found that several strains showed reduced susceptibility. Furthermore, we have shown that increased ergosterol content confers increased ethanol tolerance; a strain with a UPC2 gain of function mutation yielding higher ergosterol levels and chemical stimulation of ergosterol production both yielded increased ethanol tolerance. Likely another contributing factor in the ethanol tolerance and altered fluconazole susceptibility of these strains are alterations in chromosome copy number as all strains have aneuploidies. Future studies will continue to determine the effect of these phenotypes on a mammalian host and the importance of various transcriptional networks in these phenotypes.
Functional redundancy among members of an expanded gene family in *Candida albicans*

Emily Simonton, Matthew Z. Anderson

The Ohio State University, USA

Abstract

In eukaryotes, the regions adjacent to the ends of linear chromosomes (subtelomeres) undergo rapid evolution via elevated rates of base substitutions, indels, and recombination between homologs or with other chromosomes. These processes often give rise to lineage-specific gene family expansions, which are associated with organismal-specific adaptation and fitness across eukaryotes, including environmental and pathogenic yeasts. The *Candida albicans* telomere-associated (*TLO*) gene family increased from one or two homologs in most *Candida* spp. to 14 paralogs in the SC5314 genome reference strain and reside in subtelomeres of the eight nuclear chromosomes. *TLO* genes are the most expanded gene family in *C. albicans*, suggesting a role in host niche specialization potentially linked to commensalism and pathogenesis. Tlo proteins function as interchangeable subunits of the major transcriptional regulatory complex Mediator. However, it is not known if *TLO* paralogs retain functional redundancy, especially in the context of a large gene family expansion where multiple genes emerge within a constrained functional space. To assess the redundancy and/or partitioning of *C. albicans* *TLO* function, we will assemble a panel of single-gene knockout strains for phenotyping and transcriptional profiling to determine the impact of single-paralog loss. We anticipate that individual *TLOs* will have partially overlapping functions, with unique phenotypes arising for some deletions. Preliminary phenotypic data indicates a lack of redundancy in specific phenotypes, such as filamentation and biofilm formation for *TLOβ2*. Understanding the function of individual *TLO* paralogs will resolve potential trajectories for functional innovation in lineage-specific gene expansions and the importance of *TLOs* in adaptation.
Cdc14 phosphatase contributes to cell wall integrity and pathogenesis in *Candida albicans*

Mark Hall¹, Kedric Milholland¹, Ahmed AbdelKhalek¹, Kortany Baker¹, Smriti Hoda¹, Andrew DeMarco¹, Antonio Esperilla-Munoz², Jaime Correa-Bordes², Sanjeev Narayanan³, Scott Briggs¹

¹Purdue University, USA. ²Universidad de Extremadura, Spain. ³Purdue, USA

Abstract

Life-threatening invasive fungal infections are an escalating problem due to increasing numbers of immune-compromised individuals, the evolution of drug resistance in pathogens, climate change, and other factors. New molecular targets are needed to spur antifungal drug development to address this problem. My lab has become interested in the Cdc14 protein phosphatase as a potential antifungal target. Cdc14 is highly conserved in the fungal kingdom and was previously shown to be important for host infection by several phytopathogens through an unknown mechanism. Here, I will present our recent evidence that Cdc14 is also crucial for virulence in *Candida albicans*. Importantly, even partial loss of Cdc14 function renders *C. albicans* hypersensitive to echinocandin drugs and perturbs cell wall damage signaling, revealing a novel function for the Cdc14 family in promoting cell wall integrity that appears conserved across Ascomycota. Cdc14-deficient *C. albicans* also exhibit aberrant primary septa during cell division, which may contribute to the cell wall integrity phenotype, and are defective in hyphal formation, particularly in invasive growth conditions and a mouse infection model. I will discuss how the strict and conserved substrate specificity of fungal Cdc14 enzymes makes them amenable to development of highly selective inhibitors that could be effective against diverse pathogens.
MSS2 maintains mitochondria function and is required for chitosan resistance, invasive growth and virulence in C. albicans.

Cai-Ling Ke, Ching-Hsuan Lin
National Taiwan University, Taiwan

Abstract

Candida albicans is the most prevalent fungal pathogen in humans, particularly in immunocompromised patients. In this study, we first identified that MSS2 gene, an MSS2 ortholog in Saccharomyces cerevisiae required for mitochondria respiratory, is involved in the chitosan resistance via screening the C. albicans mutant library. Under chitosan treatment, the growth of mss2Δ strains was striking impaired but MSS2 expression was significantly inhibited by chitosan. Furthermore, mss2Δ strains were unable to grow on a medium supplemented with glycerol as the sole carbon source. Similar to the chitosan-treated wild-type strain, the ATP production ability was significantly impaired in mss2Δ. Together, these results demonstrated that an antifungal effect of chitosan against C. albicans is mediated via inhibition of MSS2 and mitochondrial biogenesis. It has been shown that normal mitochondria function is required for virulence in C. albicans. In addition, the MSS2 deletion strains showed invasive growth defect and were unable to form robust biofilms, resulting in significantly reduced virulence in a murine model. Interestingly, we found that mss2Δ mutant strains exhibited significantly impaired invasive growth ability on solid agar, but not in liquid medium. RNA-seq was therefore performed and 194 genes expressed significantly. Among them, 46 have been knocked out. The invasive ability of each mutant is under investigation. Together, these studies will allow us to clarify the mechanical relationship between mitochondria and invasive hyphal growth and understand what specific key components control cellular behaviors of invasive hyphal growth in C. albicans.
Ileal mucus inhibits hyphal *C. albicans* colonization and modulates fungal fitness in the gut.

Kuo-Yao Tseng¹, Wei-Lin Chen¹,², Yu-Huan Tsai¹,²

¹Institute of Microbiology and Immunology, National Yang-Ming Chiao Tung University, Taipei, Taiwan, Taiwan. ²Program in Molecular Medicine, National Yang-Ming Chiao Tung University and Academia Sinica, Taipei, Taiwan, Taiwan

Abstract

*Candida albicans* is the main fungal commensal in the gut in healthy individuals, and a deadly pathogen causing systemic infections from the gut in immunocompromised patients. This dimorphic fungus can present yeast and hyphal morphotypes in the host, where the hyphae have been demonstrated to express an array of virulence factors but exhibit lower fitness in gut colonization. Intestinal mucus has been shown to be pivotal in maintaining the homeostasis of intestinal mucosa. However, the role of intestinal mucus in modulating the fitness of *C. albicans* in the gut and their interaction with mucosal tissues remain unexplored. We demonstrate that oral gavage of *C. albicans* rapidly induced mucus secretion in the small intestine but not in the colon in mice as soon as six hours post inoculation. Using the yeast-locked (*EFG1/CPH1Δ*) and the hyphae-locked (*TUP1Δ*) *C. albicans* mutants, we demonstrate that the secreted ileal mucus specifically associated to the hyphae and promotes the transit of the hyphal cells to the colon. We further isolated the mouse ileal mucus and demonstrated that the ileal mucus inhibited the attachment of *C. albicans* hyphae to epithelial cells and biofilm formation, but had no significant impact on the yeast cells. We labelled the isolated ileal mucus with fluorescence-conjugated CFSE and showed that the ileal mucus preferentially bound to the hyphae, which was dependent upon hydrophobicity-mediated protein-protein interactions. Our findings reveal that ileal mucus specifically decreases the colonization of virulent hyphae to prevent intestinal tissue damage.
Dissection of the *Candida albicans* secretome

Maria Cortada Roca, Elaine Bignell, Duncan Wilson, Steven Bates

MRC Centre for Medical Mycology, United Kingdom

Abstract

The fungal secretome is uniquely important to pathogenic lifestyles, providing a range of factors that directly interact with the host. The human fungal pathogen *Candida albicans* releases a number of proteins into the extracellular environment that contribute to central virulence. However, despite its importance, relatively few of the proteins *C. albicans* is predicted to secrete have been characterised. In this study, we aim to identify and characterise the secreted proteins required for virulence. Through *in silico* analysis, we have identified a total of 135 putative classically secreted proteins, including 82 grouped across 24 families and 53 singletons. The gene families identified include proteins with known or predicted functions in nutrient acquisition and cell wall remodelling. Previously characterised proteins include hydrolytic enzymes, such as secreted aspartyl proteinases, lipases, phospholipases, and arginases, as well as a range of enzymes involved in cell wall remodelling. Uncharacterised families include esterases, sphingolipid phosphodiesterases, ribonucleases and nucleoside scavengers, plus 5 families that lack any known functional domains. Of the 53 singletons very few have been characterised to date, but those previously studied include Ece1, Hex1, Pho100, Kre9, Ecm14, Pra1 and Sel1. Moreover, 31 predicted singletons have no recognised functional domains. We have generated a library of barcoded null mutants in the predicted singletons, using the CRISPR/Cas9 transient system, and we are currently characterising their role in disease. Our work will therefore contribute to have a better understanding of the role of secreted factors in fungal virulence, and could ultimately provide novel targets for antifungal drugs and vaccines.
High-throughput phenotypic and genotypic screening and its association with antifungal resistance and carbon utilization in 379 Candida parapsilosis strains

Evelyn Zuniga, Sean Bergin, Conor Hession, Jillian Scully, Adam Ryan, Geraldine Butler

University College of Dublin, Ireland

Abstract

Candida parapsilosis is currently identified as the second or third species of Candida isolated from patients that poses a significant threat among immunosuppressed patients admitted in intensive care units (ICU). Little is known about the association between the genetic variation and the phenotypic response in terms of antifungal resistance and carbon utilization among different C. parapsilosis isolates/strains. To date 379 isolates collected from different geographic locations have been fully sequenced and screened against 25 antifungals, 8 stressing agents and 13 different carbon sources. Preliminary data of high throughput phenotypic analysis suggests that there is an evident phenotypic variation on the metabolization of antifungals and carbon utilization among the 379 C. parapsilosis strains indicated by the difference in LogRatio (colony growth) between samples. Our aim is to assess how different levels of metabolization of the different strains tested can correlate with genomic data allowing the identification of potential gene clusters among phenotypically related strains. This study will allow us narrow down and hopefully identify novel potential genes associated with antifungal resistance and expand our knowledge on carbon utilization in C. parapsilosis. Once the potential genes have been identified we will use genome editing with CRISPR-Cas9 as an initial proof of concept.
Fire Ant Alkaloids Inhibit Growth of *Candida auris*

Leandro Honorato¹, Zamith-Miranda Daniel², John Bonilla¹, Alessandro Valdez¹, Joshua Nosanchuk², Eduardo Fox³, Leonardo Nimrichter¹

¹Federal University of Rio de Janeiro, Brazil. ²Albert Einstein College of Medicine, USA. ³State University of Goias, Brazil

Abstract

The spread of *Candida auris* across the world has been closely monitored since the first description of the species in 2009. Due to a remarkably high frequency of antifungal-resistant strains and the tenacity of its biofilm, *C. auris* is considered as an urgent threat by the CDC, being the only fungus in this category, as well as a species of critical importance by the WHO. Given the current limitations with antifungal therapy for *C. auris*, the identification of new drugs is of paramount importance. Alkaloids isolated from the fire ant (*Solenopsis invicta*), also known as solenopsins, inhibit bacteria biofilms. In this work, we tested fire ant alkaloids against *C. auris* biofilm and planktonic cells. Solenopsins inhibited the growth of two fluconazole-resistant *C. auris* strains in a planktonic setting, alone and synergistically with amphotericin B. The alkaloids also inhibited matrix deposition and cellular viability of *C. auris* in biofilm conditions. Based on propidium iodide incorporation by alkaloid-treated *C. auris* planktonic cells, membrane integrity is affected by solenopsins. Additionally, treatment of *Galleria mellonella* larvae with the alkaloids promoted control of candidiasis in concentrations that are not toxic for these invertebrates. Hence, solenopsins are a potential new weapon for combating *C. auris* and our work highlights how natural compound screening can lead to the identification of new antifungal compounds.
Investigating the Role of the *TLO* genes in the White-Opaque Switching Program of *Candida albicans*

Leenah Alaalm, Derek J Sullivan, Gary P Moran

Department of Oral Biosciences, Dublin Dental University Hospital, Trinity College Dublin, Ireland

Abstract

Background

*Candida albicans* can exist as part of the normal microbiome but can become a pathogen when host immunity is compromised. This requires a rapid change of its transcriptional activity, which is controlled by the essential and conserved Mediator complex. The Mediator complex consists of 25 subunits distributed between four modules: kinase, head, middle, and tail. The *TLO* gene family encoding Med2, one of the tail’s subunits, has been noticeably expanded in *C. albicans* in comparison to other species, reaching 15 orthologues. This suggests that it plays a role in the virulence of this organism.

Methods

To study the role of *TLO* genes in virulence in *C. albicans*, we employed the CRISPR system to generate a strain in which the 15 *MED2* orthologues have been deleted in a Mating Type Locus (MTL) heterozygous background.

Results

Global gene expression analysis of a Δtlo mutant showed an increase in expression of the major white-opaque switching regulators, suggesting a role in white-opaque switching. Previous studies in a MTL homozygous background showed that deletion of subunits of the tail Mediator module other than Med2, affected the white-opaque switching in both directions. Our results showed that the Δtlo mutant did not switch unless it was homozygous at the MTL. Furthermore, mass spectrometry analysis showed Tlo interacts with the other tail subunits Med3 and Med15. Deleting *MED3* or *MED15* abolished the white to opaque switching, while the Δtlo mutant was able to switch but showed non-typical sectors or opaque colonies.
Cadmium-induced cross resistance to fluconazole in *Candida albicans*

William Burris, Agnes Zurek, Aaron Neumann

Department of Pathology, School of Medicine, University of New Mexico, Albuquerque, NM 87131, USA

Abstract

Chronic dietary exposure to heavy metals can occur through contaminated drinking and agricultural irrigation water, exposing both the host and its microbiome to metal-induced stress. Microbial adaptation to metal stress can result in cross-resistance to antimicrobials, but this has not been studied in *Candida*. We hypothesized that adaptation to cadmium stress would result in cross-resistance to fluconazole. We generated a cadmium-adapted *C. albicans* strain (CdA), which grew in the presence Cd concentrations inhibitory to control strain (SC5314). CdA cells exhibited enlarged morphology, abnormal cell wall structure, hyperadhesion and altered hyphal germination. Biofilm growth of control SC5314 *C. albicans* was significantly inhibited by fluconazole. In contrast, CdA *C. albicans* showed no dose dependent inhibition by fluconazole, although CdA biofilms did grow more slowly. These data indicate that Cd-induced stress caused antifungal cross-resistance in *C. albicans*. We were able to measure uptake of Cd into SC5314 yeast, suggesting that the metal was being sequestered in the cell. We found that mitochondrial content is increased in CdA *C. albicans* yeast relative to controls, and further increased in CdA stressed with Cd. We propose that Cd adaptation may poise cells to engage energy-dependent mechanisms that allow cross-over persistence in the face of toxic concentrations of multiple chemical threats. This mechanism may promote endogenous antimicrobial resistance (AMR) in the microbiomes of individuals with chronic metal exposure, increasing the risk of AMR infection after trauma or invasive medical procedures.
Heightened efficacy of anidulafungin when used in combination with manogepix or 5-flucytosine against *Candida auris* in vitro

Larissa L. H. John¹, Darren D. Thomson¹, Alistair J. P. Brown¹, Tihana Bicanic²,¹, Thomas S. Harrison²,¹, Elaine Bignell¹

¹Medical Research Council Centre for Medical Mycology at the University of Exeter, United Kingdom. ²Institute of Infection and Immunity, St George’s University of London, United Kingdom

Abstract

*Candida auris* is an emerging, nosocomial pathogen associated with multi-drug resistance and high mortality rates. The limited availability of antifungal drugs complicates treatment. Antifungal combination therapies hold the potential for increased efficacy through additive or synergistic impacts and might delay the emergence of resistance. The combinatorial impacts of amphotericin B, voriconazole, anidulafungin, 5-flucytosine and manogepix against 11 *C. auris* isolates (55 combinations) exhibiting a spectrum of antifungal susceptibilities were investigated in vitro using checkerboard assays. The combinations were analysed using the fractional inhibitory concentration index (FICI) and response surface analysis. Combinations of 5-flucytosine with amphotericin B, voriconazole or manogepix resulted mainly in indifferent interactions with median FICI ranging from 0.58-1.01, 0.82-2.76 and 0.56-4.41, respectively. However, the combinations of anidulafungin with 5-flucytosine or manogepix displayed synergistic interactions for 4/11 and 5/11 isolates, respectively. The high potency of these anidulafungin combinations against a multidrug-resistant isolate was confirmed using live-cell microfluidics-assisted imaging of fungal growth. Both combinations significantly increased the doubling times and markedly reduced the size of colonies in the presence of the drug combinations compared to the individual antifungals. Decreases in colony size of 63.5 % (anidulafungin-5-flucytosine) and 96.5 % (anidulafungin-manogepix) were observed after 24 h compared to 5-flucytosine and manogepix alone, respectively. The findings of this study suggest significant potential for novel combination therapies to treat multidrug resistant isolates of *C. auris*. 
Tumorous and non-tumorous oral epithelial cell responses to Candida species at the transcription regulatory level

Renata Toth, Marton Horvath, Attila Gacser

University of Szeged, Hungary

Abstract

Our recent findings suggest that Candida albicans enhances the progression of oral squamous cell carcinoma. Our aim is to reveal what are the underlying mechanisms, particularly, the post-transcriptional regulatory processes contributing to this phenomenon.

As we have previously shown that different Candida species trigger distinctive host responses from non-tumorous oral epithelial cells at the transcriptional level, we now aimed to assess whether a 'species-specific' effect could be also observed in tumorous oral epithelial cells. Under non-tumorous conditions, the species-specific host response manifested in C.albicans inducing strong inflammatory processes, while C.parapsilosis activated responses independent of inflammation, but related to carbohydrate metabolism, vascularization, and hypoxia. The identified miRNA profiles also revealed a species-specific effect. In case of tumorous oral epithelial cells a species-specific response could also be detected. After C.albicans stimulus a higher number- and different species- of miRNAs showed an altered expression, compared to C.parapsilosis, and besides inflammation, general signaling pathways related to TF and receptor regulator activity were also altered. C.parapsilosis triggered host responses similar to non-tumorous epithelial cells. Comparison of non-tumorous and tumorous conditions revealed that the number of differently expressed genes was highest after C.albicans stimulus and the majority of cancer-associated genes altered in the tumorous condition. Based on miRNA-target mRNA analyses, 6 C.albicans-induced oral tumor-associated genes were identified under miRNA regulation, that will be in the focus of our further investigations.

To summarize, tumorous oral epithelial cells also actively discriminate between C.albicans and C.parapsilosis, and the pro-tumor effect of C.albicans is regulated at the miRNA level.
Characterization of activity and mechanism of action of the *Enterococcus faecalis* bacteriocin EntV on fungal pathogens

Giuseppe Buda De Cesare, Shantanu Guha, Melissa Cruz, Shane Cristy, Antonino Zito, Michael Lorenz, Danielle Garsin

Dept. of Microbiology and Molecular Genetics, Univ. of Texas McGovern Medical School at Houston, Texas, USA

Abstract

Treatment of fungal infections is complicated by limited antifungal options and the development of drug resistance. Previous work from our group demonstrated that the bacterium *Enterococcus faecalis*, a normal constituent of the oral and gut microbiome that is often co-isolated with *Candida albicans*, antagonizes hyphal morphogenesis, biofilm formation, and virulence in *C. albicans*. These effects are mediated by EntV, a 68aa bacteriocin produced by *E. faecalis*. Based on structural data, we identified a 12aa fragment of EntV that was fully active in both in vitro and in vivo experiments, including mouse oropharyngeal candidiasis and disseminated infection models. It also protects against *Cryptococcus neoformans*, drug-resistant *C. albicans*, and *Candida auris*. Given the promising results, we are currently investigating the mechanism of action of the EntV peptides using several approaches. The 12mer localizes to the cell surface, binding more avidly to hyphae compared to yeast cells, suggesting the peptide target is more abundant in hyphae. The peptide colocalizes with dyes that preferentially stain extracellular vesicles in *C. albicans* and *C. neoformans*. In fact, EVs are involved in virulence and biofilm formation in multiple fungal species and the similar localization of EntV in both species suggests a conserved mechanism of action. Lastly the 12mer induces transcriptome changes indicating possible metabolic and cell wall remodelling mechanisms associated with peptide exposure. Together these approaches are working to identify the molecular mechanism of EntV and to enhance its activity, both important steps in its further development as a potential therapeutic.
Systematic Genetic Analysis of *Candida albicans* Filamentation in Response to Elevated Temperature

Emma Lash\(^1\), Victoria Prudent\(^2\), Corinne Maufrais\(^3\), Peter J Stogios\(^1\), Alexei Savchenko\(^{1,4,5}\), Guilhem Janbon\(^3\), Suzanne M Noble\(^2\), Nicole Robbins\(^3\), Leah E Cowen\(^1\)

\(^1\)University of Toronto, Canada. \(^2\)University of California San Francisco, USA. \(^3\)Institut Pasteur, France. 
\(^4\)University of Calgary, Canada. \(^5\)Center for Structural Genomics of Infectious Diseases, USA

Abstract

The ability to transition between yeast and filamentous growth states is critical for virulence of the leading human fungal pathogen *Candida albicans*. Large-scale genetic screens have identified hundreds of genes required for this morphological switch, but the mechanisms by which many of these genes orchestrate this developmental transition remain elusive. We screened a mutant collection covering ~40% of the *C. albicans* genome and identified 39 genes required for filamentation at 39 °C, conditions relevant to febrile episodes in the host. In this study, we characterized the role of Ent2 in governing morphogenesis in *C. albicans*. We showed that Ent2 is required for hyphal morphogenesis under a wide range of inducing conditions and is also required for virulence in a mouse model of systemic candidiasis. We found that the epsin N-terminal homology (ENTH) domain of Ent2 enables morphogenesis and virulence and does so via a physical interaction with the Cdc42 GAP Rga2 and regulation of its localization. Further analyses revealed that overexpression of the Cdc42 effector protein Cla4 can overcome the requirement for the ENTH-Rga2 physical interaction, indicating that Ent2 functions at least in part to enable proper activation of the Cdc42-Cla4 signaling pathway in the presence of a filament-inducing cue. The functional genomic screen results also suggested a role for mRNA splicing in mediating morphogenesis and further work will elucidate how these processes are connected. Overall, this work illuminates genes important for morphogenesis in response to high temperature and advances our understanding of the factors important for *C. albicans* pathogenicity.
Impact of storage on stability and biological functions of extracellular vesicles released by *Candida albicans*.

Alessandro Valdez\(^1,2\), Leandro Honorato\(^1\), Jhon J. A. Bonilla\(^1\), Flavia C. G. Reis\(^3\), Marcio L. Rodrigues\(^3,1\), Joshua D. Nosanchuk\(^2\), Leonardo Nimrichter\(^1\)

\(^1\)Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Brazil. \(^2\)Departments of Medicine (Division of Infectious Diseases) and Microbiology and Immunology, Albert Einstein College of Medicine, USA. \(^3\)Centro de Desenvolvimento Tecnológico em Saúde (CDTS), Fundação Oswaldo Cruz, Brazil

Abstract

Extracellular vesicles (EVs) released by *Candida albicans* (Ca) are multi-antigenic compartments that regulate virulence as they efficiently protect insects and mice against lethal candidiasis. Additionally, lipid components of Ca EVs modulate yeast-to-hyphae differentiation resulting in modifications of biofilm formation and fungal virulence. The dramatic effects of Ca EVs demonstrate that they are, for example, important new tools for vaccine development, active therapy, and further explorations of disease pathogenesis. However, the stability, appropriate storage and handling conditions for Ca EVs are largely unexplored. In this study, we evaluated the stability of Ca EVs isolated using three distinct protocols and subjected the EVs to different storage conditions, including long-term storage, temperature variation and vacuum concentration. Transmission electron microscopy (TEM) revealed that EV preparations maintained morphological stability at 4 °C and -20 °C for 7 days with or without vacuum concentration as well as after long-term (4 years) storage at -80 °C. However, at room temperature (RT), TEM showed that the vesicles lost membrane conformity. Furthermore, all of the Ca EVs maintained their biological properties as demonstrated by their ability to protect *G. mellonella* against *C. albicans* infection and inhibit yeast-to-hyphae differentiation, even with RT where EV morphological changes were observed. Overall, our findings demonstrate the remarkable morphological and biological stability of Ca EVs under several conditions, and this information allows investigators to more efficiently and reproducibly leverage these multi-antigenic structures for research and, potentially, as therapeutics.
Immunological fitness of echinocandin-resistant *C. glabrata*

Louise BASMACIYAN\(^1,2\), Nidia ALVAREZ-RUEDA\(^3\), Delphine ALDEBERT\(^4\), Fabienne BON\(^1\), Pascale WINCKLER\(^1\), Patrice LE PAPE\(^3\), Muriel CORNET\(^4\), Frédéric DALLE\(^1,2\)

\(^1\)UMR PAM Univ Bourgogne Franche-Comté - AgroSup Dijon - Equipe Vin, Aliment, Microbiologie, Stress, Dijon., France. \(^2\)Laboratoire de Parasitologie-Mycologie, Plateforme de Biologie Hospitalo-Universitaire Gérard Mack, Dijon., France. \(^3\)Nantes Université, CHU de Nantes, Cibles et médicaments des infections et de l’immunité, IICiMed, UR1155, F-44000 Nantes, France. \(^4\)Laboratoire TIMC IMAG ThéReX, CNRS UMR 5525, Grenoble, France

Abstract

The human fungal pathogen *Candida (C.) glabrata* can rapidly develop resistance to echinocandins, resulting in microbiological and clinical failure. Today, the impact of *C. glabrata* antifungal resistance on the host immune response remains poorly understood. Indeed, the cell wall of the fungus is involved in the recognition of *C. glabrata* by the host cells by establishing the first contact with the host tissues. However, most mechanisms of antifungal resistance involve direct or indirect modification of the fungal cell wall. Thus, mutations associated with echinocandin resistance may be responsible for enhancing the fitness cost of clinical isolates. Fitness attributes, such as immune evasion, may be altered in these resistant isolates, altering the interaction between the fungi and the host.

Here, we present an analysis of the immunological fitness of echinocandin-resistant isolates using a series of *C. glabrata* isolates that developed resistance to caspofungin in a patient treated for recurrent candidemia in blood.

First, we confirmed that echinocandin resistance was indeed associated with changes in the composition of the fungal cell wall. Then, using in vitro yeast-macrophage and yeast-human leukocyte co-culture models, we showed that the induced immune response differed between susceptible and resistant isolates. Particularly, we report that caspofungin-resistant *C. glabrata* isolates survive significantly within immune aggregates and induce reduced and delayed secretion of pro-inflammatory cytokines compared to susceptible isolates.

All in all, this study suggest that immune persistence may be considered an additional fitness attribute involved in the pathophysiological interaction of caspofungin-resistant *C. glabrata* isolates with the host.
Variations in the composition of the intestinal microbiota and mycobiota in laboratory mice and effects on colonization with *Candida albicans*

Sarah Vielreicher¹, Wibke Krüger¹, Cristina Zubiría Barrera², Tilman Klassert², Hortense Slevogt², Katja Schubert¹, Rakesh Santhanam³, Gianni Panagiotou³, Ilse D. Jacobsen¹, ², ³, ⁴

¹Microbial Immunology, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Germany. ²Dynamics of Respiratory Infections, Helmholtz Centre for Infection Research, Germany. ³Systems Biology and Bioinformatics, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Germany. ⁴Institute of Microbiology, Friedrich Schiller University Jena, Germany

Abstract

The resident bacterial microbiota restricts *Candida albicans* proliferation in the gut by conferring colonization resistance. In this context, microbial dysbiosis induced by antibiotic treatment represents a major risk factor for candidiasis.

Aiming to identify bacteria mediating colonization resistance, we investigated natural microbiota variation in laboratory mice. Based on 16S and ITS sequencing of fecal samples from 20 C57BL/6 breeding colonies, differences in α- and β-diversity, and quantitative microbial burden were used as criteria to select 5 colonies for colonization experiments. The animals received different antibiotics or no treatment and a single oral administration of *C. albicans*. Fungal burden was monitored in feces on different time points up to 14 days post-colonization. Further, microbial loads within specific intestinal organ tissue and content were analyzed at end of experiment.

All tested colonies showed similar colonization patterns despite microbiome variations. The highest level of fungal colonization was found in mice receiving a combination of antibiotics. Surprisingly, stable colonization with up to $1 \times 10^5$ *C. albicans* CFU/g in feces and gut was detected even in the absence of antibiotic treatment. Sucrose supplementation of drinking water, used to increase acceptance of antibiotic-containing drinking water, was identified as promoting *C. albicans* colonization in the absence of antibiotics.

Our results show that considerable microbiota variation in mouse breeding colonies does not necessarily affect colonization resistance. Additionally, the data set can be used for the detection of bacterial candidates for colonization resistance in the future.
Candidalysin drives Candida albicans induced-IL-1β expression and release during oral infection

Alexander Kemp, Giorgio Camilli, James S. Griffiths, Julian Naglik

Centre for Host-Microbiome Interactions, Faculty of Dentistry, Oral and Craniofacial Sciences, King’s College London, United Kingdom

Abstract

Candida albicans is a common fungal pathogen, causing a multifaceted disease phenotype, including life-threatening invasive and disseminated infections (especially in immunocompromised individuals), as well as mucocutaneous disorders. During infection, candidalysin, a secreted hyphal toxin, is crucial for epithelial cell damage as well as for initiating the mucosal immune response. In both disseminated and oral C. albicans infections, the potent pro-inflammatory cytokine Interleukin (IL)-1β orchestrates the activation of innate and adaptive immune cells to mediate a protective host response. To generate the bioactive cytokine, pro-IL-1β requires cleavage, either by a multiprotein caspase-1-activating complex termed the inflammasome, or inflammasome-independent enzymatic processes. We demonstrate that candidalysin is vital for IL-1β expression and secretion by human oral epithelial cells. Furthermore, we show that the synthesis of pro-IL-1β in oral epithelial cells depends on EGFR and EPHA2 activation by C. albicans. Consolidating our in vitro results, in an acute oropharyngeal candidiasis model we observe elevated levels of pro- and mature IL-1β in oral tissues only after infection with candidalysin-producing C. albicans strains. Interestingly, cell death and the activation and secretion of IL-1β were not reduced by employing pharmacological inhibition of NLRP3 inflammasome or caspases in vitro, consistent with observing no caspase-1 activation in mouse tongues or human oral epithelia during infection. Collectively, our findings illustrate the dependence of epithelial cell damage, IL-1β synthesis and secretion on candidalysin and C. albicans and suggest an alternative and inflammasome/caspase-independent pathway of IL-1β activation.
Investigation of the RNA interference pathway in *Candida albicans*

Elise Iracane, Cristina Arias-Sarda, Alessia Buscaino

University of Kent, United Kingdom

**Abstract**

RNA interference mechanism has been studied in the fission yeast *Schizosaccharomyces pombe* and has been found in the budding yeast *Saccharomyces castellii*, but it is not present in *Saccharomyces cerevisiae*. In *Candida albicans*, the main actors Argonaute (Ago1) and a non-canonical Dicer (Dcr1) are present, but no active RNAi pathway has been described.

In this study we compared genomic data publicly available to find differences between *C. albicans* isolates. Out of 30 different isolates, we found that only in the lab strain SC5314, Ago1 is mutated in a conserved residue important for the protein’s function in *Drosophila melanogaster*. We used CRISPR-Cas9 method to delete AGO1 or reverse the mutation in several *C. albicans* strains. Using the same method, we also mutated DCR1 to remove the last double-strand RNA binding domain in the same isolates.

In the isolate P94015, RNA-seq of small RNA showed a large quantity of sRNA in the telomeric regions, including at *TLO* genes. The number of sRNA is highly reduced in the DCR1 mutant, suggesting the involvement of Dcr1 in the sRNA synthesis process. Moreover, using qPCR analysis, *TLO* genes expression is increased in ago1 mutants of three isolates compared to their WT, except in SC5314. These results suggest that Ago1 is involved in silencing of *TLO* genes in *C. albicans*, but that Ago1 in SC5314 seems to be inactive. I will present the results of this ongoing analysis.
**In vitro and in vivo experimental evolution expose a broad diversity of acquired amphotericin B resistance mechanisms and fitness trade-off compensation in Candida auris**

Hans Carolus\textsuperscript{1}, Dimitrios Sofras\textsuperscript{2}, Poppy Sephton-Clark\textsuperscript{2}, Siebe Pierson\textsuperscript{1}, Celia Lobo Romero\textsuperscript{1}, Rudy Vergauwen\textsuperscript{1}, Jacques F. Meis\textsuperscript{3}, Jeffrey M. Rybak\textsuperscript{4}, Christina A. Cuomo\textsuperscript{2}, Patrick Van Dijck\textsuperscript{1}

\textsuperscript{1}Laboratory of Molecular Cell Biology, Department of Biology, KU Leuven, Leuven, Belgium. \textsuperscript{2}Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA. \textsuperscript{3}Centre of Expertise in Mycology Radboudumc/CWZ, Nijmegen, Netherlands. \textsuperscript{4}St. Jude Children’s Research Hospital, Memphis, Tennessee, USA

**Abstract**

*Candida auris* is a recently emerged human fungal pathogen of growing concern due to common (multi)drug resistance. Although it is the first fungal pathogen to be officially considered an urgent antimicrobial resistance threat by CDC, resistance mechanisms have only begun to be studied. Notably resistance to amphotericin B, present in 40 to 60% of all clinical isolates of *C. auris*, remains largely uncharacterized.

This is the first large scale investigation of amphotericin B resistance in *C. auris*, in which we have typed 441 in vitro (serial transfer) and in vivo (systemic mouse model) evolved *C. auris* lineages from four parental strains of genetically diverse clades. We show a great diversity of acquired resistance responses with resistance magnitude- and clade dependent fitness trade-offs. Genotyping and membrane sterol analyses of selected lineages show membrane sterol alterations due to mutations in genes involved in sterol biosynthesis including *ERG6, NCP1, ERG3, ERG11, HMG1, ERG10* and *ERG12*. Whole genome sequencing identified additional novel resistance mechanisms, including chromosomal aneuploidies, alterations in oxidative stress tolerance, iron homeostasis, sphingolipid and sterol biosynthesis regulation. Furthermore, fitness characterization revealed mechanisms of fitness trade-off compensation, a phenomenon unexplored in pathogenic fungi.

A comparative genome analysis of 773 clinical isolates of *C. auris* showed that a number of strains show allelic variability in genes of interest identified in our study. Overall, the novel mechanisms of resistance and fitness compensation as identified here can pose a significant clinical threat to be taken into consideration in the fight against *Candida auris*. 
Biofilm-protein kinase connections in *Candida albicans*

Minju Kim¹, Juraj Kramara², Tomye Ollinger², Aaron Mitchell¹, Damian Krysan²

¹University of Georgia, USA. ²University of Iowa, USA

Abstract

*Candida albicans* can grow as a biofilm community on implanted medical devices or mucosal surfaces, thus driving infection. Biofilm biology is complex; biofilm growth alters the expression of as many as 1500 genes compared to free-living planktonic growth. Two biological processes are known to be required for biofilm formation: adherence and hypha formation. Adherence under most conditions depends upon ALS gene products, especially the highly expressed family member ALS3. Hypha formation under most conditions depends upon *HGC1*, a hypha-associated cyclin gene that promotes polarized growth through multiple targets.

Our study addresses the roles of protein kinases (PKs) in biofilm formation. PKs are often signaling hubs that connect multiple pathways. We have created a set of 104 bar-coded PK gene deletion mutants and screened them for biofilm defects in a 96 well-plate format. We found that *cbk1Δ/Δ* and *kic1Δ/Δ* mutants make only nominal biofilms that lack hyphae. To test the functional basis for their defects, we created derivatives of each mutant that express either ALS3 or *HGC1* from the strong *RBT5* promoter. Both derivatives of these mutants have increased biofilm formation ability, though neither produces as much biofilm biomass as the wild type. Rescue by ALS3 expression can be rationalized by its impact on adherence. However, the functional basis for rescue by *HGC1* expression is not as apparent. This system offers the opportunity to explore this connection between biofilm formation, hypha formation, and PK-dependent signaling.

*M.K. and J.K. are co-first authors.*
The Candida Genome Database (CGD, http://www.candidagenome.org/) serves as an online compendium of information about Candida albicans SC5314 and other Candida species, including C. glabrata, C. parapsilosis, C. dubliniensis, and now C. auris. CGD maintains the most current genome sequences and manually collects published experimental data that include mutant phenotypes and functional characteristics of gene products captured as Gene Ontology (GO) annotations. Since the last Candida and Candidiasis Meeting, CGD has made major upgrades. We added the species Candida auris due to its emergence as a major public health threat, with rapid spread and high levels of drug tolerance. We have to date added just over 12,000 Gene Ontology annotations for C. auris. Other major upgrades are more computational, including a new server with greater speed and reliability and replacement of the sequence browser GBrowse with JBrowse, which has the flexibility to incorporate more types of data. With the ability to add tracks for conservation and expression data that were not formerly available, we welcome datasets for all five species. The sequence and functional information are seamlessly integrated with multiple analysis tools at the CGD website. The website further serves as a hub for meeting announcements, job opportunities, and news about Candida research. CGD staff is pleased to help with specific needs or questions and we appreciate the continued community support. Reach us at candida-curator@lists.stanford.edu.

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The cell wall glucan-glycogen complex: a novel determinant of the *C. albicans* host-pathogen interaction

Jian Miao¹, Bridget Graves², Zuchao Ma², Douglas Lowman², Tammy Ozment², Nicole Lewis², Michael Kruppa², David Williams², Brian Peters¹

¹University of Tennessee Health Science Center, USA. ²East Tennessee State University, USA

Abstract

Complex carbohydrates are major components of the fungal cell wall and serve as important pathogen associated molecular patterns (PAMPs) to potentiate innate immune responses. We recently reported that glycogen and β-(1→3)-glucan form a covalently linked macromolecular complex associated with the *C. albicans* cell wall. Challenge of human PBMCs with isolated glucan-glycogen complexes led to strong increases in pro-inflammatory cytokine signaling (e.g., IL-1β, IL-6, TNF-α) as measured by ELISA and transcriptional profiling. Additional studies revealed that altered glucan-glycogen ratios impact immune stimulation and that these particles must be covalently linked to exert potent cytokine release.

Furthermore, using genetic and biochemical approaches, we confirmed that loss of *GSY1* (glycogen synthase) or *GLC3* (branching enzyme) ablated cell wall glycogen content. Challenge of human macrophages with formalin-fixed *gsy1Δ/Δ* or *glc3Δ/Δ* led to exacerbated secretion of IL-1β as compared to WT or revertant strains. Analysis of cell wall components by fluorescence staining and flow cytometry revealed that levels of total glucan (aniline blue), total mannan (concanavalin A) and total chitin (calcofluor white) remained similar, while reduced glycogen (iodine staining) significantly correlated with increased β-(1→3)-glucan (monoclonal antibody) exposure. Moreover, a collection of clinical isolates was screened for glycogen content. Remarkably, all isolates with reduced glycogen were hyperinflammatory during macrophage challenge and demonstrated augmented β-(1→3)-glucan display. Overexpression of *GSY1* in a subset of these isolates reversed their hyperinflammatory phenotype. Collectively, our data demonstrate that the glucan-glycogen complex may be a novel fungal PAMP important for governing the host-*Candida* interaction.
Injectable fat emulsion (IFE) choice impacts *Candida* biofilm formation and hyphal growth via a *UME6*-dependent mechanism

Gustavo Alvira-Arill, Hubertine Willems, Jabez Fortwendel, Jeremy Stultz, Brian Peters

University of Tennessee Health Science Center, USA

**Abstract**

Major differences exist between IFEs and their ability to support fungal biofilm growth. Mixed-oil IFE (MO-IFE; SMOFlipid®) was previously shown to inhibit *C. albicans* biofilm and the yeast-to-hypha switch in vitro due to capric acid within this formulation. To delineate the inhibitory mechanism, *C. albicans* was cultured for 4 h in minimal medium (MM) or MM supplemented with 10% soybean-oil IFE (SO-IFE; Intralipid®), 10% MO-IFE, or 10% SO-IFE supplemented with capric acid. Total RNA was extracted, sequenced, and differential gene expression analyses conducted. Genes found significantly downregulated in both MO-IFE and SO-IFE + capric acid included transcriptional regulators, cyclins, and septins associated with hyphal growth, including: *UME6*, *HGC1*, and *CDC11*. Over-expression of these targets were accomplished using a doxycycline-repressible promoter system and each strain tagged to constitutively express GFP. In the absence of doxycycline, PrtetO-*HGC1* and PrtetO-*UME6* formed hyphae during growth in MO-IFE or capric acid as observed by light or fluorescence microscopy. However, doxycycline-mediated repression of ectopic expression of *HGC1* or *UME6* led to significantly reduced hyphal growth. As expected, hyphal growth was not impaired under any conditions in SO-IFE. Over-expression of *UME6* (but interestingly not *HGC1*) was sufficient for rescuing biofilm growth in MO-IFE. Moreover, growth in MO-IFE or capric acid led to frequently observed clusters of incompletely separated cells that displayed abnormal chitin deposition by calcofluor white staining, indicating impaired cytokinesis. These studies support further investigation on the impact of IFE choice on clinical incidence of fungal biofilm-mediated infections, such as catheter-related bloodstream infections.
A051

SR-like RNA-binding protein 1 affects transport of ASH1 mRNA in *Candida albicans* hyphae

Emma Beane¹,², Kate Paulsen¹,³, Yi Peng Wang¹,⁴, Anne McBride¹

¹Bowdoin College, USA. ²Duke University, USA. ³Stanford University School of Medicine, USA. ⁴Stanford University, USA

**Abstract**

Transport of mRNAs facilitates local translation at sites of protein function and polarized processes. Polarized hyphal growth of the opportunistic pathogenic fungus *Candida albicans* is linked to virulence. The absence of either cytoplasmic mRNA-transport protein CaShe3 or predominantly nuclear SR-like RNA-binding protein 1 (Slr1) affects hyphal growth and function. A mutant Slr1 protein localizes to the hyphal tip; intriguingly, mutant Slr hyphal tip localization decreases in the absence of She3. We aim to test whether Slr1 is involved in She3-mediated mRNA transport in C. albicans by determining: 1. whether Slr1 interacts with She3 and She3-transported mRNAs, using immunoprecipitation and reverse transcription-PCR, and 2. whether SLR1 deletion impacts ASH1 mRNA localization to the hyphal tip, using fluorescence in situ hybridization. A fraction of FLAG-tagged She3 co-immunoprecipitated from hyphal cell lysates with Slr1-GFP. The She3-transported ASH1 mRNA was enriched in Slr1-GFP-bound RNA pools and enrichment of ASH1 is greater for the hyphal-tip localized mutant slr1-GFP than for wildtype Slr1-GFP. Non-transported ACT1 mRNA was not significantly enriched in Slr1-GFP-bound RNA pools, nor were She3-transported RBT4 and SAP5 mRNAs. Hybridization with a set of 40 ASH1-specific fluorescent probes revealed decreased hyphal tip localization of ASH1 mRNA in cells lacking Slr1. ASH1 mRNA levels were not significantly decreased in slr1Δ/Δ compared to WT hyphae. Decreased ASH1 mRNA hyphal tip localization in slr1Δ/Δ cells, combined with the physical interaction of Slr1 with She3 and ASH1 mRNA, suggests that Slr1 influences, but is not required for, transport of ASH1 mRNA to the hyphal tip.
Identifying putative hyphal regulators of *Candida albicans*

**Dakota Archambault, Michael Lorenz**

University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences, University of Texas Health Science Center Department of Microbiology and Molecular Genetics, Houston, TX, USA

**Abstract**

*Candida albicans* is a normal resident of the human microbiota, but defects in the innate immune response can permit *C. albicans* to disseminate throughout the body, leading to multisystem organ failure and carries a patient mortality rate of ~40%. How *C. albicans* interacts with phagocytes is thus relevant for the development of infection due to the complex and dynamic relationship of *C. albicans* and host macrophages. The transition to hyphal growth is a key feature of this interaction, as it contributes to macrophage damage and escape. Using transcriptomic data from several Candida species before and during phagocytosis, two sets of uncharacterized genes were identified as candidate virulence factors. The first set of genes was expressed at a significantly higher level in phagocytosed cells compared to controls and we have generated a mutant library to validate these genes for host-relevant phenotypes. Screening this library in solid and liquid media conditions has identified several candidate hyphal regulators, such as C2_04280W_A, C6_02450W_A, and CR_07170W_A. The second set of genes are more highly expressed in *C. albicans* compared to other less virulent species. This set was highly enriched for hyphal-associated genes and includes an uncharacterized homolog of the yeast Dig2 morphogenetic regulator, which is annotated as an essential gene. We are generating genetic tools to study this uncharacterized homolog, C3_01800C, to determine its essentiality and impact on hyphal growth. In this study, we are probing these sets of novel genes for novel regulators of morphogenesis and virulence.
Uridine auxotrophy in *Candida albicans* disrupts cell wall architecture but attenuates virulence by slowing growth in mice

Mikayla Mangrum\(^1\), Andrew Wagner\(^1\), Jian Miao\(^2\), Elise Phillips\(^1\), Brian Peters\(^2\), Todd Reynolds\(^1\)

\(^1\)University of Tennessee, Knoxville, USA. \(^2\)University of Tennessee Health Science Center, USA

Abstract

UDP-glucose and UDP-N-acetylglucosamine are important for cell wall construction as they are the precursors for β-1,3-glucan and chitin respectively. Both products are derived from uridine, and previous studies have demonstrated attenuated virulence of uridine auxotrophs in mouse infections, which has been attributed to insufficient uridine levels to maintain growth in the host. We have discovered that uridine deprivation in the uridine auxotrophic mutant *ura3Δ/Δ* disrupts cell wall architecture by increasing surface mannans and exposing β-1,3-glucan and chitin. This can be rescued with uridine supplementation. This may be related to effects on UDP-sugar levels. The cell wall architectural changes in a *ura3Δ/Δ* mutant appear to impact immune activation since the mutant elicited greater TNFα secretion from RAW264.7 macrophages than wildtype. Here we used a murine model of systemic infection which showed mice infected with a *ura3Δ/Δ* mutant exhibit increased survival and reduced kidney fungal burden compared to mice infected with wildtype *C. albicans*. Suppression of the immune response with cyclophosphamide did not decrease survival nor increase kidney colonization of *ura3Δ/Δ* infected mice, indicating the attenuation in virulence of uridine auxotrophs can be attributed to decreased fitness in the host but not increased exposure of β-1,3-glucan. Moreover, a *ura3Δ/Δ* mutant is unable to grow on ex vivo kidney agar which demonstrates its inability to colonize the kidneys due to poor growth. Thus, although uridine auxotrophy elicits changes to cell wall architecture that increase the exposure of immunogenic epitopes, this exposure does not fully explain the attenuation in virulence observed in uridine auxotrophs.
Culturomics analysis of gut mycobiota in patients with ulcerative colitis and characterization of *Candida albicans* isolates

Piyapat Rintarhat¹, Daehwan Kim², Daniel Croll³, Do Yup Lee²,⁴,⁵,², Won Hee Jung¹

¹Department of Systems Biotechnology, Chung-Ang University, Korea, Republic of. ²Department of Agricultural Biotechnology, Seoul National University, Korea, Republic of. ³Laboratory of Evolutionary Genetics, Institute of Biology, University of Neuchâtel, Switzerland. ⁴Center for Food and Bioconvergence, Seoul National University, Korea, Republic of. ⁵Research Institute for Agricultural and Life Sciences, Seoul National University, Korea, Republic of

Abstract

The human gut is colonized by diverse microorganisms, including bacteria, viruses, protozoa, and fungi. Several studies have suggested that the gut fungal microbiota (mycobiota) impacts the host immunity and the development and progression of human diseases. However, most gut microbiota studies have focused exclusively on bacteria, and the mycobiota in the organ have largely been unexplored. Here, we developed a culturomics platform to isolate the fungal strains from fecal samples of a cohort of Korean patients with ulcerative colitis (UC) and compared them with those of healthy subjects (HT). Our culturomics analysis showed that most identified fungal colonies belonged to the phylum Ascomycota followed by Basidiomycota both in the HT subjects and UC patients. The total number of colonies from the fecal samples of the UC patients was significantly higher than that of the HT subjects, suggesting that more fungal strains may persist in the intestines of the UC patients compared to that of the HT subjects. Moreover, we collected some *Candida albicans* isolates, which was one of the most dominant fungal species in the fecal samples. The phenotypic and genotypic characteristics of the *C. albicans* isolates from fecal samples were analyzed and compared with that of the same fungal species isolated from the different niches such as the gut mucosal layer and blood. The results of the comparisons between the different *C. albicans* isolates are presented. Our study emphasizes the importance of the gut mycobiota and provides useful information on *C. albicans* residing in the human gut.
The role of the fungal virulence factor Ece1 in homeostatic immunity towards 
*Candida albicans*

Julia Lagler\(^1\), Ricardo Fróis-Martins\(^1\), Meret Tuor\(^1\), Osama Elshafee\(^2\), Selene Mogavero\(^2\), Bernhard Hube\(^3\), Salomé LeibundGut-Landmann\(^1\)

\(^1\)Section of Immunology, Vetsuisse Faculty, and Institute of Experimental Immunology, University of Zurich, Switzerland. \(^2\)Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology, Hans Knoell Institute, Germany

Abstract

The peptide toxin candidalysin is a key virulence factor of *C. albicans* inducing damage in host cells and promoting inflammation in infected tissues. Candidalysin is also promoting IL-17 production in an acute oral candidiasis model. The role of the toxin during commensalism however, where it is expressed at low levels, remains unclear. Here, we investigated whether candidalysin is involved in driving homeostatic Th17 immunity in the *C. albicans*-colonized oral mucosa, which is required for long-term maintenance of commensalism. For this, we assessed the fungus-specific Th17 response of mice that were experimentally colonized with a commensal strain of *C. albicans* deficient or sufficient for the candidalysin-encoding gene *ECE1*.

Th17 immunity in the oral cavity and in the draining lymph nodes (drLN) was strongly decreased in mice colonized with an *ECE1*-deficient commensal isolate of *C. albicans* in comparison to the wildtype control strain. This contrasted with the Th17 response to a high-virulent and rapidly cleared strain of *C. albicans*, which was found to be *ECE1*-independent, indicating that *ECE1*-dependence differs between high- and low-virulent strains. The observation that dendritic cell (DC) maturation and migration from the mucosal tissue to drLN was also found reduced during commensal colonization, although independently of direct recognition of candidalysin by DCs, provide insights into the mechanisms of *ECE1*-dependent Th17 priming.

In conclusion, Ece1 is required for driving homeostatic Th17 immunity during commensal *C. albicans* colonization while Ece1-independent factors can compensate for the induction of Th17 cells during acute OPC with the high-virulent strain.
Escherichia coli Nissle 1917 antogonizes Candida albicans growth and protects intestinal cells from C. albicans-mediated damage

Yasmine REBAI1,2, Lysett WAGNER3, Mayssa GNAIEN1,2, Merle HAMMER3, Mario KAPITAN3, Maria Joanna NIEMIEC3, Helmi MARDASSI1,2, Slavena VYLKOVA3, Ilse JACOBSEN3, Sadri ZNAIDI1,4

1Institut Pasteur de Tunis, Tunisia. 2University of Tunis El Manar, Tunisia. 3Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Germany. 4Institut Pasteur Paris, France

Abstract

Candida albicans is a pathobiont of the gastrointestinal tract. It can contribute to the diversity of the gut microbiome without causing harmful effects. When the immune system is compromised, C. albicans can damage intestinal cells and cause invasive disease. We hypothesize that a therapeutic approach against C. albicans infections can rely on the antimicrobial properties of probiotic bacteria. We investigated the impact of probiotic strain Escherichia coli Nissle 1917 (EcN) on C. albicans growth and its ability to cause damage to intestinal cells. In co-culture kinetic assay, C. albicans abundance gradually decreased over time compared to C. albicans abundance in the absence of EcN. Quantification of C. albicans survival suggests that EcN exerts a fungicidal activity. To test if an EcN-released compound maybe responsible for C. albicans growth inhibition, C. albicans doubling times were determined during growth in cell-free supernatants (cfs) collected from both monoculture and co-culture assays. While EcN monoculture cfs did not affect C. albicans growth, co-culture cfs mildly decreased it, suggesting that EcN’s activity may depend on the interaction of both species. Using a model of co-culture in the presence of human gut epithelial cells, we further show that EcN prevents C. albicans from exerting its cytotoxic effect on enterocytes. Consistently, both C. albicans filamentous growth and microcolony formation were altered by EcN. Taken together, our study proposes that probiotic strain EcN can be exploited for future therapeutic approaches against C. albicans infections.

Keywords: Escherichia coli; Nissle 1917; probiotics, Candida albicans, polymicrobial interactions
Phosphoinositide 3-kinase signalling regulates trafficking of plasma membrane transporters in *Candida glabrata*

FIZZA ASKARI

CENTRE FOR DNA FINGERPRINTING AND DIAGNOSTICS, India

Abstract

*Candida (Nakaseomyces) glabrata*, one of the causative agents of invasive candidiasis, has recently been listed as a high-priority fungal pathogen by the World Health Organization. In cases of invasive candidiasis, *C. glabrata* accounts for the mortality rate of approximately 40%. Noteworthy, as *C. glabrata* is resistant to azoles, a major class of antifungal drugs, treatment is more challenging, thus imposing a higher risk of mortality. The flexibility of pathogenic fungi to obtain and utilize various nutrient sources depending on the host niche is essential for their survival, proliferation, and virulence. Consequently, one prospective technique for antifungal treatment is to target the nutrient transport mechanism. In this work, we demonstrated that the retrograde trafficking of several plasma membrane transporters is dependent on a sole phosphoinositide 3-kinase (*CgVps34*). To understand the molecular mechanism of *CgVps34*-dependent retrograde trafficking, we chose *CgFtr1*, a high affinity iron transporter protein, and analysed its trafficking from the plasma membrane to the vacuole in response to excessive extracellular iron conditions. We have employed genetic, proteomic, biophysical, and microscopic techniques and identified a novel effector of phosphoinositide 3-phosphate (PI3P), *CgPil1* protein. We have proposed that the amount of PI3P on the plasma membrane controls the interaction of *CgPil1* with two important lysine residues in the C-terminus of *CgFtr1*, functioning as a sorting station for the vacuolar transport of *CgFtr1*. This molecular mechanism of *CgFtr1* trafficking will be discussed in depth.
Riboflavin (vitamin B2) production in *Candida albicans*: An interesting antifungal drug target but is it also an underlying reason for the commensal lifestyle of this fungus.

Jana Nysten¹, Wouter Van Genechten¹, Rudy Vergauwen¹, Wim Van den Ende², Saleh Yazdani¹, Liesbeth Demuyser¹, Patrick Van Dijck¹

¹KU Leuven Laboratory of Molecular Cell biology, Belgium. ²KU Leuven Laboratory of Molecular Plant Biology, Belgium

Abstract

Riboflavin is a yellow pigment, also termed vitamin B2. Unlike animals, fungi can synthesize this essential component themselves, thereby leading us to appreciate that targeting riboflavin production is a promising novel strategy against fungal infections. We recently showed that the GTP cyclohydrolase encoded by *C. albicans* RIB1 (CaRIB1) is essential and rate-limiting for production of riboflavin in the fungal pathogen. We confirm the high potential of CaRib1 as an antifungal drug target, as its deletion completely impairs in vivo infectibility by *C. albicans* in model systems. Besides being an interesting antifungal drug target, the capacity to produce riboflavin, may also be the reason why so many people do have *C. albicans* in their gut or on other mucosal layers. *C. albicans* (and other microbes) may provide humans with the necessary vitamin B2 as this vitamin is an essential micronutrient involved in cellular metabolism. As it cannot be synthesized endogenously by the human host, it needs to be obtained through the diet. We now show in mice that *C. albicans* produces excess riboflavin which the mice can absorb. A *C. albicans* RIB1-overexpressing strain prevents ariboflavinosis, an extreme riboflavin deficiency, in a mouse gastrointestinal colonization model. Furthermore, this strain has a competitive advantage compared to a wild type strain in the small intestine where dietary riboflavin is predominantly absorbed by the host. This beneficial effect may provide evolutionary pressure for the establishment and maintenance of this opportunistic pathogen in the human gut.
"Pour some sugar on me" – Candida albicans evolves increased virulence potential and antifungal resistance via adaptations to host dietary sugars

Theresa Lange1, Jakob Sprague1, Raghav Vij1, Silvia Radosa1, Falk Hillmann2, Sascha Brunke1, Bernhard Hube1,3

1Hans Knoell Institute Jena, Germany. 2Hochschule Wismar, Germany. 3Friedrich Schiller University, Germany

Abstract

The opportunistic pathogen Candida albicans is normally found as a commensal that colonizes human mucosal surfaces of the oral cavity, vagina, and gastrointestinal tract. However, in 2016 three of the first environmental C. albicans isolates were found on old oak trees far from urban influences (Robinson et al. 2016).

We characterized the virulence potential, growth, and antifungal susceptibility of these strains. One strain exhibited a surprisingly high level of damage to epithelial cells and is intrinsically resistant to amphotericin B. We propose that this phenotype emerged from natural interactions in an "environmental virulence school" and are currently investigating the confrontation of the C. albicans oak tree isolates with environmental amoebae.

We also analyzed host-associated factors that would allow the environmental C. albicans isolates to adapt to, colonize, and infect humans. In a long-term evolution experiment in sugar-rich medium, we observed that a less virulent oak tree isolate increased its metabolic flexibility and damage potential toward epithelial cells, presumably becoming better adapted to the human host. Furthermore, this sugar-adapted strain developed unexpected resistances to antifungals.

These data suggest that nutritional triggers in the western diet, such as sugars, can affect C. albicans’ virulence and antifungal resistances through evolution within the host. The changes of the oak tree strain on the genetic and transcriptomic level during adaptation to the sugar-rich medium are being investigated to determine the causes for the increased virulence and antifungal resistance.
Targeting Polyamine Synthesis and Transport in *Candida albicans*

Arya Vindu¹, Brendan Snarr², Chune Cao¹, Ronda J. Rolfes³, Michail S. Lionakis², Otto Phanstiel IV⁴, Thomas E. Dever¹

¹Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA. ²National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD 20892, USA. ³Department of Biology, Georgetown University, Washington, D.C. 20057, USA. ⁴College of Medicine, University of Central Florida, Orlando, FL 32826, USA

Abstract

Polyamines, small organic polycations, are essential for life, and cells maintain polyamines via synthesis and uptake. In eukaryotes, the three major polyamines, putrescine, spermidine and spermine, are implicated in important cellular functions including transcription, mRNA translation, and oxidative stress protection. Recently, we identified Hol1 as the high-affinity polyamine transporter in *S. cerevisiae*. Hol1 is a conserved fungal-specific transporter with *Candida albicans* having two *HOL1* homologs (orf19.4889 and orf19.2991) and no identifiable *HOL1* homolog in mammals. As polyamines are essential, we hypothesized that polyamine depletion via combined inhibition of polyamine biosynthesis and uptake would impair growth and virulence of *C. albicans*. Consistent with this hypothesis, simultaneously treating cells with L-α-difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase (ODC), to inhibit polyamine biosynthesis and with synthetic polyamine analogs to inhibit uptake substantially impaired *C. albicans* growth. Deleting both *HOL1* homologs blocked efficient polyamine uptake in *C. albicans*, establishing Hol1 as the high-affinity polyamine transporter in *C. albicans*. Combined deletion of *HOL1* and *SPE1*, encoding ornithine decarboxylase, resulted in a severe growth defect, confirming the importance of polyamines for *C. albicans* growth. In the mouse model of disseminated candidiasis, whereas the *spe1*-/− mutant displayed similar virulence to the isogenic WT strain with mice dying within 2 weeks following intravenous inoculation, the *spe1*-/− *hol1*-/− mutant was avirulent with all mice surviving the infection. Thus, we conclude that polyamines are critical for *C. albicans* virulence and could be of potential therapeutic interest via combined targeting of polyamine synthesis and the fungal-specific polyamine transporter Hol1.
A Structure-Guided Approach to Identify Fungal-Selective Yck2 Inhibitors

Bonnie Yiu\(^1\), Emily Puumala\(^1\), Pascal Marchand\(^2\), Luke Whitesell\(^1\), Nicole Robbins\(^1\), Leah Cowen\(^1\)

\(^1\)Department of Molecular Genetics, University of Toronto, Canada. \(^2\)Cibles et médicaments des infections et de l’immunité, IICiMed, Nantes Université, France

Abstract

On a global scale, fungal pathogens are responsible for \(\sim\)13 million infections and 1.5 million deaths per year. With the widespread emergence of antimicrobial resistance and the limited arsenal of antifungal therapeutics, there is a need to identify novel antifungals. Yck2 is a fungal member of the casein kinase 1 (CK1) family that governs Candida albicans pathogenesis. Chemical screens revealed imidazo[1,2-x]azine derivatives as inhibitors of Yck2, resulting in fungal-selective growth impairment in host-relevant conditions and enhancement of antifungal efficacy. However, this compound suffers from poor pharmacological properties. To determine the potential of Yck2 inhibitors as a novel class of antifungal, I utilized genetic and biochemical approaches to characterize nine newly synthesized imidazo[1,2-x]azine derivatives. Using a kinase assay to measure casein kinase inhibition, I showed several derivatives displayed fungal-selective activity against the fungal isoform Yck2 compared to mammalian CK1a. In a standard dose-response assay, I identified CTN1756 and CTN1844 as the most bioactive molecules against C.albicans. CTN1756 and CTN1844 demonstrated on-target whole-cell activity as a conditionally-repressible yck2 mutant was hypersensitive to compound inhibition. Further, compound treatment resulted in polarized growth in C.albicans, a phenotype consistent with Yck2 inhibition. Furthermore, I showed that CTN1756 and CTN1844 enhanced caspofungin efficacy in an echinocandin-resistant strain. Unfortunately, all compounds demonstrated low metabolic stability in a mouse liver microsome assay. Future work will focus on the generation of additional imidazo[1,2-x]azine derivatives to further optimize the potency, selectivity, and metabolic stability of the scaffold to optimize Yck2 inhibitors as a novel class of antifungal.
Antioxidant pathways that protect the *Candida albicans* plasma membrane

Kara Swenson, James Konopka
Stony Brook University, USA

Abstract

The *Candida albicans* plasma membrane (PM) is on the front line of attack by reactive oxygen species (ROS) generated by the immune system. ROS can cause a chain reaction known as lipid peroxidation which disrupts the integrity of the PM and creates a variety of lipid radicals and peroxides, resulting in widespread damage. Therefore, the *C. albicans* PM must have special pathways to protect against oxidation. Our lab found that Flavodoxin-Like Proteins (Pst1, Pst2, Pst3, and Ycp4) protect against lipid peroxidation and are essential for virulence in mice, which highlighted the importance of protecting membrane lipids from oxidation. In other organisms, such as mammals, Glutathione Peroxidases (GPxs) detoxify lipid peroxides to promote PM integrity and ROS resistance. *C. albicans* has four GPxs (Gpx3, Gpx31, Gpx32, and Gpx33), therefore we created a quadruple mutant lacking all four GPx genes and found that it was very sensitive to organic peroxides, including lipid peroxides. However, a gpx3Δ deletion mutant had about the same level of sensitivity to oxidative stress as the GPx quadruple mutant, and Gpx3 is known to regulate Cap1, a major transcription factor for inducing ROS resistance genes. Furthermore, a cap1Δ mutant showed a similar level of sensitivity to peroxides as the gpx3Δ and quadruple mutant strains. Surprisingly, this indicates that GPxs primarily regulate Cap1 and do not play a direct role in protecting against lipid peroxidation. Future research will focus on the genes regulated by Cap1 that promote resistance to organic and lipid peroxides.
The calcineurin subunit Cna1 is a positive regulator of transcription factor Pdr1 and azole resistance in Candida glabrata

Bao G. Vu, Lucia Simonicova, Scott Moye-Rowley
University of Iowa, USA

Abstract

The calcineurin subunit Cna1 is a positive regulator of transcription factor Pdr1 and azole resistance in Candida glabrata

Vu B.G., Simonicova L., and Moye-Rowley, W.S.
Department of Molecular Physiology and Biophysics, Carver College of Medicine, University of Iowa, Iowa City, Iowa, USA.

Background: Transcription factor Pdr1 is essential in azole resistance of Candida glabrata. Gain-of-function (GOF) mutations are often found in Pdr1 among hyper-resistant clinical isolates. In this study, we show that the phosphatase function of calcineurin protein is required for Pdr1 expression and normal azole tolerance in C. glabrata.

Methods: Previous work found that Pdr1 and its target gene, encoding the ATP-binding cassette transporter Cdr1, were both induced in response to challenge with fluconazole (FLC). However, the upstream regulation of Pdr1 remains unknown. Mass spectrometric analyses of Pdr1- partners identified Cna1 as potential regulator. CNA1 alleles, including null and enzymatically defective mutants, were used to examine the effects of phosphatase on azole resistance. Analyses of Pdr1/Cdr1 expression in both wild type and strains carrying PDR1-GOF mutations by western and RNA measurements demonstrated that Cna1 was required for normal expression and regulation of these genes.

Results: Cna1 is required for wild-type levels of Pdr1 expression along with FLC-triggered induction of CDR1. Loss of Cna1 phosphatase function inhibits Pdr1 and Cdr1 expression, as well as azole resistance in C. glabrata.
Conclusions: These findings detail the significant role of calcineurin pathway in C. glabrata azole resistance and suggest a connection between the efflux pump system and cell wall synthesis in Candida species.
Uncommon Candida species causing nosocomial candidaemia in paediatrics with malignancies in Iran

Fatemeh Ahangarkani¹, Mohammad Sadegh Rezai², Hamid Badali³, Kiana Abbasi⁴

¹Antimicrobial Resistance Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran, Iran, Islamic Republic of. ²Pediatric Infectious Diseases Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran, Iran, Islamic Republic of. ³Department of Molecular Microbiology & Immunology South Texas Center for Emerging Infectious Diseases The University of Texas at San Antonio, San Antonio, Texas, U.S., USA. ⁴Department of Microbiology, Zanjan Branch, Islamic Azad University, Zanjan, Iran., Iran, Islamic Republic of

Abstract

Background: Although Candida albicans has been mainly responsible for the majority of hospital-acquired candidaemia cases, candidemia due to non-albicans Candida species is increasing, which is associated with increased mortality and reduced susceptibility to antifungal drugs.

Methods: This observational cross-sectional study was conducted during 2017-2019 in the north of Iran. Fungal isolates were identified using matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF). Antifungal susceptibility testing was performed using the Clinical and Laboratory Standards Institute broth microdilution guideline.

Results: Candida albicans (49%), followed by C. guilliermondii (10.9%), C. tropicalis (9%), C. parapsilosis (7.27%), C. orthopsilosis (6.36%), C. dubliniensis (3.63%), C. fabianii (3.6%), C. glabrata (2.72%), C. krusei (2.72%), C. utilis (1.8%), C. kefyr (0.9%), C. eremophila (0.9%) and C. melibiosica (0.9%) isolated from 108 malignancies children with candidemia. Fluconazole exhibited the highest MIC50 and MIC90 (64 μg/mL) values against C. tropicalis, followed by C. glabrata. All fluconazole-resistant isolates were non-albicans Candida, that is C. tropicalis (6.5%), with MIC > 64 μg/mL; C. glabrata and C. krusei (1.8% for both), with MIC 32 μg/mL; and C. orthopsilosis and C. eremophila (0.9% for both), with MIC 16 μg/mL. Remarkably, anidulafungin and micafungin demonstrated the lowest MIC90 values (0.031 μg/mL) against C. tropicalis.

Conclusion: Uncommon Candida species with reduced susceptibility to antifungals are emerging as significant agents of nosocomial candidaemia in high-risk paediatric patients in Iran.
Natural variation in hemin-induced filamentation among *Candida albicans* isolates

Liping Xiong

University of Georgia, USA

Abstract

*Candida albicans* can use external hemin as an alternative to elemental iron. The Martinez lab reported that hemin also specifically acts as an inducer of filamentation (PMID: 9317050). However, the mechanism by which hemin promotes filamentation and morphological change is not known. We investigated this phenomenon among 26 diverse *C. albicans* isolates and found natural variation in response to hemin. In addition, we observed that hemin supplementation improves biofilm formation of its strong responders. In *C. albicans* type strain SC5314, investigation of transcriptional response showed that hemin induced a typical filamentation response that includes many known hypha-associated genes. We verified that this filamentation response depends on well-characterized filamentation regulators. Furthermore, we determined that the genes *ZCF20* and *HMX1* are specifically induced by additional hemin. *ZCF20* encodes a zinc finger transcription factor recently shown by the Kornitzer lab (PMID: 36084128) to be an activator of heme uptake and homeostasis genes. *HMX1* encodes a heme oxygenase that defends against free heme toxicity (Lesuisse lab; PMID: 1263432). We find that HMX1 is required for biofilm formation under exposure to excess hemin. The basis for variation in the response to hemin remains uncertain.
The Ras-cAMP-PKA pathway regulates *Candida albicans* hyphae to lateral yeast production

M. Amin ul Mannan¹, Maikel Acosta-Zaldívar², Eliciane Mattos¹, Julia R. Köhler², Priya Uppuluri¹

¹Division of Infectious Diseases, The Lundquist Institute at Harbor-UCLA Medical Center, Torrance, CA, USA. ²Division of Infectious Diseases, Boston Children’s Hospital/Harvard Medical School, Boston, MA, USA

Abstract

*Candida albicans* hyphae produce yeast cells laterally on their subapical segments while apical segments continue to extend as filamentous cells. These lateral yeasts are important for proliferation of *C. albicans* in tissues during invasive disease, and critical for its spread through the bloodstream. They are dispersal cells that dissociate from biofilms and seek new substrates to infect. Little is known about the signals that induce lateral yeast growth. Our previous studies showed that high glucose or farnesol can trigger lateral yeast growth from biofilm hyphae. Here we demonstrate that both these triggers also upregulate *PES1* in hyphae, a gene previously determined to be essential for lateral yeast growth. Downregulation of Ras-cAMP components such as *RAS1, CYR1, TPK1* or *TPK2* also upregulates *PES1* in hyphae and induces lateral yeast growth. Addition of exogenous cAMP to a strain that prolifically produces lateral yeast because it constitutively upregulates Pes1, can significantly reduce lateral yeast production from hyphae. Pes1 depletion was found to be synthetically lethal in a *TPK2*, but not in a *TPK1* mutant strain, specifying the importance of protein kinase A in the regulatory network that controls this process. Together, these studies indicate that the Ras-cAMP-PKA pathway negatively regulates lateral yeast production and suggest its regulatory connection to Pes1. Studies to determine whether, during lateral yeast production, this pathway regulates Pes1 through its phosphorylation state are underway.
Sur7 regulates plasma membrane lipid asymmetry in *Candida albicans*

Kaela Teele, Lois Douglas, James Konopka
Stony Brook University, USA

Abstract

*C. albicans* can cause lethal systemic infections, especially in immunocompromised individuals, because of *C. albicans’* ability to resist stress from the host. Studies indicate that the plasma membrane (PM) play key roles in promoting stress resistance, especially the PM subdomains, eisosomes. Eisosomes are invaginations of the PM and are enriched in proteins that promote resistance to different types of stress, including copper, which is used by the immune system to fight off infections. Sur7 was found to be one of these proteins. Previous studies demonstrated that the *sur7Δ* mutant is more sensitive to copper because phosphatidylserine (PS) is mislocalized to the outer leaflet allowing copper to bind, due to copper’s high affinity to PS, and damage the PM. PS is typically enriched in the inner leaflet of the PM by phospholipid flippases that translocate PS to the inner leaflet from the outer leaflet. Consistent with this, mutation of the *DRS2* flippase resulted in a similar increase in sensitivity to copper as the *sur7Δ* mutant. To assess if PS was in the proper leaflet, we used papuamide A or copper that bind PS to evaluate the localization of this phospholipid in the *sur7Δ* and observed it mislocalized to improper leaflet. Thus, we hypothesize that Sur7 regulates flippase function and to test this, we are determining how the *sur7Δ* mutation affects the production and localization of the five different flippases in *C. albicans*. These studies will help determine how *C. albicans* resist attacks by the immune system and cause serious infections.
Structure-guided optimization of small molecules targeting the yeast casein kinase, Yck2 as a therapeutic strategy to combat Candida albicans

Emily Puumala¹, Meganathan Nandakumar², Noelle S. Williams³, Peter Stogios¹, Luke Whitesell¹, Nicole Robbins¹, Timothy M. Willson², Leah E. Cowen¹

¹University of Toronto, Canada. ²University of North Carolina at Chapel Hill, USA. ³University of Texas Southwestern Medical School, USA

Abstract

Candida albicans is a common etiological agent of candidiasis, capable of causing life-threatening systemic disease with mortality rates of ~40%. Protein kinases have been rewarding targets in drug development for diverse diseases but remain untapped in the quest for new antifungals. This study builds on the success of a screen of kinase inhibitors against a drug-resistant isolate of C. albicans, which revealed a 2,3-aryl-pyrazolopyridine molecule, GW461484A (GW) that targets a member of the casein kinase 1 (CK1) family, YcK2. C. albicans Yck2 is required for growth under physiological conditions, maintenance of echinocandin resistance, and virulence in a mouse model of infection. While the GW scaffold demonstrates potent bioactivity against C. albicans, its poor metabolic stability presents a key liability for its progression into in vivo efficacy studies. We thus engaged in medicinal chemistry efforts to optimize the GW scaffold. Two sets of molecules, GW bioisosters employing an imidazo[1,2-a]pyridine scaffold, and structure-guided R-substituents of the parent GW pyrazolo[1,5-a]pyridine scaffold, were generated. Through biochemical and microbiological testing of dozens of analogs, we determined that three key molecules demonstrated improved pharmacological properties. Specifically, our most optimal compounds demonstrated improved metabolic stability in mouse liver microsomes while retaining whole-cell bioactivity and selectivity for the fungal Yck2 compared to the human CK1 isoform, CK1α. Follow-up pharmacokinetic studies highlighted the promise of these molecules for use in a systemic model of candidiasis. Overall, this study highlights a practical route to develop a new class of antifungal, employing a previously unexploited mode of action.
Carbonate inhibits *Candida albicans* filamentation and biofilm formation.

Trenton Miedema¹, Kayla Keckler¹-², Ian Cleary¹

¹Grand Valley State University, USA. ²Western Michigan University, USA

Abstract

Growth of *Candida albicans* on implanted medical devices, such as vascular catheters, pacemakers, and artificial heart valves, is a serious problem and additional options for preventing or reducing growth on these surfaces are required. Carbonate containing compounds, such as sodium bicarbonate and potassium bicarbonate, have been used as alternatives to conventional fungicides in agricultural operations. However, there has been limited examination of the effects of carbonate on medically relevant fungi. We tested the effects of carbonate on key virulence traits of *C. albicans* including hyphal growth and biofilm formation. Adding carbonate to growth media at a concentration that did not affect the overall growth rate resulted in reduced filamentation, reduced invasion of solid media and reduced biofilm formation, suggesting that carbonate may be a useful addition in our efforts to control *C. albicans* biofilm formation on solid surfaces.
Expressing *Candida albicans* hyphal surface proteins in yeast-locked strains

Nicholas Schena, Kassandra Baker, Anna Stark, Derek Thomas, Ian Cleary

Grand Valley State University, USA

**Abstract**

A number of *Candida albicans* mutant strains have been constructed that are locked in the yeast form and which do not form hyphae when grown in inducing conditions. The cells of these strains typically do not adhere well to each other in broth cultures and the biofilms formed by these strains are loose and easily disrupted. During the change from yeast to hyphae, expression of surface proteins characterized as adhesins is upregulated and a loss of the genes encoding such proteins can affect the adhesive properties of hyphae. Here, we examine the effect on adhesion and biofilm formation of expressing some of these surface proteins in yeast-locked strains. We have observed improvements in biofilm formation and adhesion to glass slides without any alteration in the morphology of the cells. Interestingly, there is variation between the strains, indicating that there are differences in the cell surface between the different yeast-locked strains.
Identification and characterization of molecules with novel antifungal activity against *Candida albicans*

Sara Fallah¹, William Steinbach², Joseph Heitman³, John Porco⁴, Luke Whitesell¹, Lauren Brown⁴, Partha Nag⁵, Nicole Robbins¹, Leah Cowen¹

¹Department of Molecular Genetics, University of Toronto, Canada. ²College of Medicine, University of Arkansas, USA. ³Department of Molecular Genetics and Microbiology, Duke University, USA. ⁴Department of Chemistry and Center for Molecular Discovery (BU-CMD), Boston University, USA. ⁵Chemical Biology and Therapeutics Science (CBTS), The Broad Institute of MIT and Harvard, USA

Abstract

Infection with *Candida albicans*, one of the most prevalent fungal pathogens of humans, causes a diverse range of diseases extending from superficial infections to deadly systemic mycoses. Currently, only three major classes of antifungal drugs are available to treat systemic infections: azoles, polyenes, and echinocandins. Alarmingly, the efficacy of these antifungals against *C. albicans* is hindered both by basal tolerance towards the drug and the development of resistance mechanisms such as alterations of the drug’s target, modulation of stress responses, and overexpression of efflux pumps. My research focuses on the identification and characterization of compounds with novel activity against *C. albicans*. Leveraging the Boston University Center for Molecular Discovery (BU-CMD)’s chemical library, I screened 3,280 compounds against a *C. albicans* clinical isolate and identified 16 molecules that inhibit *C. albicans* growth through metal chelation. Media supplementation with ferric or ferrous iron rescued *C. albicans* growth, indicating these compounds exert their antifungal activity primarily through iron chelation. Furthermore, I characterized the mode of action of two compounds with novel antifungal activity from the Broad Institute Diversity-Oriented Synthesis (DOS) library. Through genetic approaches, I identified one molecule as an inhibitor of Erg11, despite the compound’s lack of a canonical azole ring, and another compound as a translation inhibitor. Future work will leverage biochemical methods to further define compound mode of action and focus on investigating the therapeutic potential of prioritized molecules. Overall, my research has identified compounds with novel antifungal activity that may have potential for much needed future drug development.
Lipid flippases in *Candida albicans*

Miguel Basante-Bedoya, Stephanie Bogliolo, Robert Arkowitz, Martine Bassilana

University Côte d'Azur, CNRS, France

**Abstract**

Flippases transport lipids across the membrane bilayer to generate and maintain asymmetry. *Candida albicans* has 5 flippases, including Drs2 that is important for filamentous growth, phosphatidylserine (PS) distribution and sensitivity to the antifungal drug fluconazole (1). This mutant has also an altered distribution of phosphatidylinositol 4-phosphate [PI(4)P] and ergosterol, and is reduced for virulence in a murine model for systemic candidiasis (2). Deletion of the oxysterol binding protein (Osh) lipid transfer protein, Osh4 in the *drs2* mutant specifically bypasses the requirement for this flippase in invasive filamentous growth, but does not restore growth on fluconazole or papuamide A, a toxin that binds PS in the outer leaflet of the plasma membrane, indicating that Drs2 has additional role(s), independent of Osh4 (2). Recent studies using point mutants in another flippase, Dnf2, indicate that transport of phosphatidylcholine and/or the sphingolipid glucosylceramide is important for filamentous growth and virulence (3). We are currently further investigating the roles of Drs2 and that of the PS bilayer asymmetry, using mutants of Drs2 as well as that of the flippase Neo1.


Abstract

Despite Candida albicans (C. albicans) being one of the most prevalent fungal pathogens, much remains undiscovered about the intricate relationship between its virulence factors, and how they function in combination to promote pathogenicity. In the host, fungal gene products work together to induce specific biological outcomes. Accordingly, phenotypes observed using single-knockout mutants may be obstructed by compensatory mechanisms.

To uncover more about the importance and relationships between virulence factors in C. albicans’ pathogenicity, CRISPR-Cas9 technology can be utilised. This can facilitate the generation of single and multi-gene knockouts in a quick and efficient manner, which can be characterised using numerous in vitro assays.

We initially focused on three virulence factors: peptide toxin candidalysin (encoded by ECE1), invasin Als3p (ALS3) and G1-cyclin Hgc1p (HGC1). Using CRISPR, single and multi-gene knockouts were generated for ECE1, ALS3 and HGC1. Deletion of ECE1 and HGC1 led to reduced epithelial cellular damage, and infection with ece1Δ/Δ also diminished cytokine production. The deletion of ALS3 in the ece1Δ/Δ mutant led to comparable damage and cytokine levels to that of the ece1Δ/Δ single-knockout, indicating candidalysin drives damage and immune activation, not Als3p. ALS3 deletion significantly impaired abiotic-surface adherence and biofilm formation, which was unaffected by the removal of ECE1 and/or HGC1. In vivo analysis of these mutants is currently ongoing.

ECE1, ALS3 and HGC1 gene-knockouts can be generated via CRISPR in C. albicans and analysis of mutants can provide insight into their combinatorial function during infection. Other combinations of virulence factors will be pursued in future studies.
Limonene inhibits virulence related traits in *Candida albicans*

SAIEMA AHMEDI

Jamia Millia Islamia, New Delhi, India

**Abstract**

**Background:** Multidrug resistance and high toxicity towards conventional antifungal drugs is causing serious concern worldwide. Exploration of novel compounds with immense therapeutic potential is required. The present study evaluates the antifungal potential of limonene, a secondary metabolite found in several citrus plants based essential oils.

**Methods:** *In-vitro* anti-*Candida* studies were performed including haemolysis assay, antifungal susceptibility, secretion of hydrolytic enzymes, morphological transition, adhesion and biofilm formation. Further validation was done by *in-silico* studies using docking tools followed by MD simulations with five major virulence associated antifungal targets (Als3, Bcr1, Plb1, Sap2 and Tec1).

**Results:** With MIC of 300 μg/ml, it causes only 1% RBC cell lysis. Limonene significantly reduced adhesion to buccal epithelial cells. At MIC, hydrolytic enzymes proteinases and phospholipases was also reduced by 73% and 53% respectively. Limonene treatment inhibits morphological transition in *C. albicans* which was monitored microscopically. Adhesion and biofilm formation was also reduced by 91% and 87% respectively. Docking and MD simulation studies confirm stable hydrophobic interactions with all the target proteins except Bcr1. Present study suggests that limonene inhibits major virulence factors in *C. albicans*.

**Conclusion:** Due to low toxicity, easy availability and high antifungal potential, limonene is a suitable candidate to be explored as an antifungal drug. Present work needs to be corroborated with molecular and *in vivo* studies to understand its mode of action and exact target sites.

**Keywords:** Limonene; Candida albicans; adhesion; biofilms; hydrolytic enzymes
Bile Acid Regulates the Colonization and Dissemination of *Candida albicans* from the Intestine

Shankar Thangamani

Purdue University, USA

Abstract

*Candida albicans* (CA) is an opportunistic fungus that frequently inhabits the gastrointestinal (GI) tract and can cause an invasive fungal infection. The microbial and host factors in the regulation of CA colonization in the intestine is poorly understood. We utilized a combination of targeted metabolomics, 16S ribosomal RNA amplicon gene sequencing, and *in vivo* mouse models of CA infection to identify the metabolites, microbiome, and host factors that regulate CA colonization in the intestine. Levels of carbohydrates, sugar alcohols and primary bile acids were increased, whereas carboxylic acids and secondary bile acids were decreased in antibiotic treated mice susceptible to CA. Among all the metabolites examined, we identified that taurocholic acid (TCA), a major bile acid present in humans and mice was significantly increased in the antibiotic treated mice susceptible to CA. *In vivo* findings indicate that administration of TCA through drinking water is sufficient to induce colonization and dissemination of CA even in the absence of antibiotic and (or) immunosuppressive treatment. TCA significantly decreased the relative abundance of three culturable species of commensal bacteria, Turicibacter sanguinis, Lactobacillus johnsonii, and Clostridium celatum in the cecal contents. Furthermore, TCA significantly reduced mRNA expression of immune genes such as ang4 and Cxcr3, decreased the number of CX3CR1+ phagocytes and T helper 17 cells that play a critical role in controlling CA in the intestine. Collectively, our results indicate that TCA modulates gut commensal bacteria and intestinal immune responses to promote CA colonization in the intestine.
**MRR1 at the intersection of drug resistance and metabolism - A snapshot of MRR1-DNA interactions in *Candida lusitaniae***

Dhanabala Subhiksha Rajesh Khanna, Elora Demers

Dartmouth College, USA

**Abstract**

Understanding the physiological consequences of mutations leading to antifungal resistance may develop new treatments for infections caused by drug-resistant strains. Gain-of-function (GOF) mutations in the gene encoding the Zn-cluster transcription factor *MRR1* mediates the overexpression of drug resistance conferring genes like the drug efflux protein encoding *MDR1* and metabolic genes like methylglyoxal reductases, *MGD1* and *MGD2*. Methylglyoxal, a spontaneous byproduct of fungal metabolism is an endogenous inducer of Mrr1. We used CUT&RUN to identify the direct targets of the *C. lusitaniae* Mrr1 to evaluate the effects of constitutive Mrr1 activity on cellular metabolism. By comparing Mrr1<sup>WT</sup>-binding profiles to our existing RNA-seq dataset, we identified 25 genes (21 upregulated and 4 downregulated) as direct targets of Mrr1. The upregulated targets included drug efflux proteins like *MDR1*, *CDR1*, and a suite of metabolic genes including *MGD1*, *MGD2*, putative aldehyde dehydrogenases and aldo-keto reductases. Syntenic blocks of these metabolic genes were also under Mrr1 regulation. We found Mrr1 peaks in distal promoter regions (≥1kB upstream of the ORF) of the highly regulated genes, indicative of regulation through cis-enhancer elements. Surprisingly, we observed no difference in the Mrr1-binding profiles between Mrr1<sup>WT</sup> and Mrr1<sup>GOF</sup> variants. Using Biolog phenotype plates, we identified a marked Mrr1<sup>GOF</sup>-dependent growth defect in glycerol and TCA cycle intermediates and impaired respiration in TTC overlay. Our studies establish *MRR1* as a mediator of both drug resistance and metabolism with a potential role in the metabolic rewiring of drug resistant isolates and in cells growing in the presence of Mrr1 inducers like methylglyoxal.
In vivo colonization and pathogenic potential of *C. africana*

Daria Kosmala¹, Natacha Sertour¹, Ricardo Martins², Salomé Leibundgut-Landmann², Luca Spaggiari³, Andrea Ardizzoni³, Eva Pericolini³, Marie-Elisabeth Bougnoux¹, Christophe d'Enfert¹, Mélanie Legrand¹

¹Institut Pasteur, France. ²University of Zurich, Switzerland. ³University of Modena and Reggio Emilia, Italy

**Abstract**

*Candida albicans* population displays high genetic diversity illustrated by 18-well differentiated genetic clusters. The most distinctive cluster of its population – Cluster 13, also known as *Candida africana* includes strains first described as atypical *C. albicans* isolates of vaginal origin and showing apparent tropism for the female genital tract. In our study, we explored colonization and pathogenic potential of *C. africana* in four *in vivo* mice models, namely gastrointestinal tract (GIT) colonization, oropharyngeal candidiasis (OPC), vulvovaginal candidiasis (VVC), and systemic candidiasis models. For our study, we selected two *C. africana* strains displaying phenotypic differences (CEC4878 and CEC4854) and compared their interactions with the host to those of reference strain SC5314 and commensal strain 529L. *C. africana* strains displayed significantly decreased ability to colonize the murine GIT compared to strains SC5314 and 529L. Moreover, in the murine model of systemic candidiasis, *C. africana* strains were unable to cause symptoms and mortality in mice, showing significantly decreased fungal burden in kidneys. While there is barely any report of *C. africana* association with the oral cavity our study revealed that *C. africana* strains can colonize the oral cavity, inducing a host immune response. Surprisingly, the VVC model revealed significant differences between the two *C. africana* strains with strain CEC4878 inducing higher host immune response. This study broadens the knowledge about *C. africana* pathogenic potential and may allow us to highlight the specific features of *C. africana* that might contribute to its apparent niche restriction.

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At least 10 genes on chromosome 5 of Candida albicans are downregulated in concert to control cell wall and to confer adaptation to caspofungin

Sudisht Sah, Anshuman Yadav, Elena Rustchenko

University of Rochester Medical Center, USA

Abstract

Candida albicans is part of normal microbiota, however, can cause superficial and life threatening infection in immune-compromised individuals. Drugs from echinocandin (ECN) class that disrupt cell wall synthesis, are being used as a major treatment strategy against candidiasis. Previously, we reported involvement of 5 chromosome 2 (Ch2) genes in adaptation to ECN drugs. Here, we explored 22 candidate-genes on Ch5 that are consistently downregulated in independent mutants adapted to caspofungin (CAS), for their role in ECN adaptation. We also compared cell wall remodeling in CAS-adapted mutants and in 10 knockouts (KOs) from Ch5. Independent KO experiments as combined with broth microdilution assay, demonstrated that, as expected, 10 out of 22 Ch5 genes decrease ECN susceptibility by controlling the levels of three major components of the cell wall, glucan, mannan, and chitin. Some KOs decreased glucan or increased chitin or both. Similar cell wall remodeling, decreased glucan and increased chitin, was found in CAS-adapted mutants with no ploidy change. Some other KOs had no glucan change, but increased the level of either mannan or chitin. Our results identify the function of two uncharacterized genes, orf19.970 and orf19.4149.1, and expand the functions of DUS4, RPS25B, UAP1, URA7, RPO26, HAS1, and CKS1. Importantly, half of the above genes are essential indicating that essential processes are involved in cell wall remodeling for adaptation to ECNs. Also important, orf19.970 and orf19.4149.1 have no human orthologues. Our work shows that multiple mechanisms are used by C. albicans to remodel cell wall for adaptation to CAS.
Candidalysin Variants Possess Different Potencies

Antzela Tsavou¹, Don N. Wickramasinghe¹, Corinne Maufrays², Christophe d'Enfert², Julian Naglik¹, Jonathan Richardson¹

¹King’s College London, United Kingdom. ²Institut Pasteur, France

Abstract

Candida albicans is a common opportunistic fungal pathogen that can exist as spherical yeast or filamentous hyphae. C. albicans hyphae are a crucial virulence determinant, particularly during mucosal infections. In addition to the yeast-to-hypha morphological transition, C. albicans possesses other virulence factors including candidalysin; a secreted peptide toxin that damages epithelial cells and activates host immunity. Candidalysin is the only currently known virulence determinant that directly drives immunopathology.

By comparing the genome sequences of 182 C. albicans isolates, we identified nine variants of candidalysin with different amino acid sequences. Here we characterised the biophysical properties and biological activity of these candidalysin variants on TR146 oral epithelial cells. Through lactate dehydrogenase (LDH) activity assays, western blotting and Luminex assays we were able to show epithelial damage, activation of MAPK signalling through EGFR, and the secretion of chemokine and cytokines, respectively. Most of these candidalysin variants possess similar potencies to the C. albicans SC5314 (reference laboratory strain) candidalysin. Interestingly, three candidalysin variants appeared to induce less cellular damage and calcium influx, decreased MAPK activation, and significantly less cytokine secretion.

Differences in amino acid sequences resulted in reduced immunostimulatory activity of these candidalysin variants. This study indicates the importance of key amino acids in governing candidalysin potency and toxicity. Candidalysin sequence variation is another contributing factor that influences the pathogenic potential of C. albicans isolates.
Candida albicans strains adapted to caspofungin due to aneuploidy become highly tolerant under continued drug pressure.

Farha Husain, Anshuman Yadav, Sudish K. Sah, Jeffrey J. Hayes, Elena Rustchenko

University of Rochester, USA

Abstract

Candida albicans is a prevalent fungal pathogen in humans. Understanding the development of decreased susceptibility to ECN drugs of this microbe is of substantial interest, as it is viewed as an intermediate step allowing the formation of FKS1 resistance mutations. We used six previously characterized mutants that decreased caspofungin susceptibility either by acquiring aneuploidy of chromosome 5 (Ch5) or by aneuploidy-independent mechanisms. When we exposed these caspofungin-adapted mutants to caspofungin again, we obtained 60 evolved mutants with further decreases in caspofungin susceptibility, as determined with CLSI method. We show that the initial adaptation to caspofungin is coupled with the adaptation to other ECNs, such as micafungin and anidulafungin, in mutants with no ploidy change, but not in aneuploid mutants, which become more susceptible to micafungin and anidulafungin. Furthermore, we find that the initial mechanism of caspofungin adaptation determines the pattern of further adaptation as parentals with no ploidy change further adapt to all ECNs by relatively small decreases in susceptibility, whereas aneuploid parentals adapt to all ECNs, primarily by a large decrease in susceptibilities. Our data suggest that either distinct or common mechanisms can govern adaptation to different ECNs.
The Arabian killifish, *Aphanius dispar*, as a novel thermo-relevant infection model to study human fungal pathogens

Tina Bedekovic, Teigan Veale, Larissa John, Atyaf Hamied, Chantelle Davies, Steven Bates, Alexandra Brand, Mark Ramsdale, Tetsuhiro Kudoh

University of Exeter, United Kingdom

Abstract

**Background:** The zebrafish embryo model has significantly advanced our understanding of fungal pathogenesis and host immune responses. However, zebrafish embryos are inviable at human body temperature. We are developing the Arabian killifish (*Aphanius dispar*) as an alternative model in which to study fungal pathogenesis at physiologically relevant temperatures.

**Methods:** The virulence of *Candida albicans* wild-type and mutant strains (including strains avirulent in a mouse model) was compared at 28 °C in zebrafish and at 30 °C and 37 °C in killifish embryos. Infection was monitored using CFUs, fluorescence microscopy and heartbeat detection. De novo genome and transcript analysis of *A. dispar* identified host immune markers, which were monitored by Western blots and qPCR.

**Results:** The rate of killing by wild-type *C. albicans* was fastest in the killifish at 37 °C and the infection was effectively treated with antifungal drugs. Importantly, mutants that were avirulent in mice and in killifish at 37 °C were virulent in zebrafish at 28 °C, suggesting lower incubation temperature has a critical impact on virulence traits. Bioinformatics revealed candidate genes related to immune function.

**Conclusion:** The Arabian killifish offers a new, genetically tractable model for understanding *C. albicans* infections and the host immune response. Its ability to thrive at 37 °C is an important advantage over zebrafish and imaging of identified immune biomarkers is now possible in the CRISPR-Cas9-generated fluorescently ‘dark’ *gch* mutant. The killifish model is being further developed and could make a significant contribution to our understanding of fungal pathogenesis.
Pyrimidine derivatives, a new strategy for collaring dual – species biofilms formed by Candida auris and Staphylococcus aureus

Vartika Srivastava1, Aijaz Ahmad2,3

1Clinical Microbiology and Infectious Diseases, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2193, South Africa. 2Clinical Microbiology and Infectious Diseases, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2193, South Africa. 3Infection Control Unit, Charlotte Maxeke Johannesburg Academic Hospital, National Health Laboratory Service, Johannesburg 2193, South Africa

Abstract

C. auris is a part of human microflora and therefore, it interacts with the other members of the skin microbiota. Staphylococcus species has evolved several tactics such as, being halotolerant and utilising urea present on the human skin. In general, Staphylococcus species are overriding the skin microbiota with S. aureus being the most abundant especially in the wound infections. Although much information on the interactive behaviour of C. auris with other microbiota is not available, we conjectured that similar to C. albicans, C. auris should intermingle with S. aureus and may contribute in increased virulence level and complicating the therapeutic regimen. In present study, we investigated the antimicrobial potential of six pyrimidine derivatives. The most effective derivative (t3) was analysed for its anti-biofilm property against mono- and poly- microbial biofilms formed by C. auris and S. aureus. Thereafter, the impact of t3 derivative on microbial viability and total biomass was studied. Followed by confocal laser scanning microscopy and Scanning electron microscopic investigation. The haemolytic activity of t3 was also determined. The MIC values recorded for t3 was 39.06 µg/ml, 19.53 µg/ml and 39.06 µg/ml against planktonic cells of C. auris, S. aureus and polymicrobial culture, respectively. The t3 derivative at sub-MIC values potentially abrogates both biofilm formation as well as 24 h mature mono-and poly- microbial biofilms. Haemolytic assay further confirmed the safety of this derivative. The results from this study advocate the anti-biofilm properties of pyrimidine derivatives against bacterial and fungal pathogens in mono- and poly- microbial biofilm condition.
Candidalysin is a key player in activating vaginal cell ROS in an in vitro infection model

Luca Spaggiari1, Andrea Ardizzoni2, Caterina Squartini-Ramos2, Gianfranco Squartini-Ramos2, Francesco Ricchi2, Elisabetta Blasi2, Francesco De Seta3, Eva Pericolini2

1Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia., Italy. 2Department of Surgical, Medical, Dental and Morphological Sciences with interest in Transplant, Oncological and Regenerative Medicine, University of Modena and Reggio Emilia., Italy. 3Department of Medical Sciences, University of Trieste, Institute for Maternal and Child Health-IRCCS, Burlo Garofolo, Trieste., Italy

Abstract

C. albicans can behave as a commensal yeast colonizing the vaginal mucosa and in this condition is tolerated by the epithelium. When the epithelial tolerance breaks down, due to C. albicans overgrowth and hyphae formation, the generated inflammatory response and cell damage lead to vulvovaginal candidiasis symptoms. Here, we studied the induction of reactive oxygen species (ROS) in vaginal epithelial cells caused by C. albicans infection and the involvement of fungal burden, morphogenesis and candidalysin (CL) production in such induction.

Wild-type C. albicans, C. albicans PCA-2 and C. albicans 529L strains were employed to infect in vitro a reconstituted vaginal epithelium (RVE), starting from A-431 cell line. ROS production was kinetically monitored by using MitoSOXTM probe. C. albicans-induced cell damage and proinflammatory cytokines production by infected cells are also being tested.

Wild-type C. albicans induced fast and high ROS production by vaginal epithelial cells, in parallel to the increase of the fungal load and to the number of hyphae. Under the same experimental conditions, the 529L C. albicans strain, known to be defective in CL production, induced a slow and scant ROS production, thus highlighting a key role of CL in causing epithelial oxidative distress. PCA-2 induced comparable but slower ROS production with wild-type C. albicans yeasts.

We conclude that CL production, more than fungal load and hyphae formation, seems to play a key role in the rapid activation of ROS by epithelial cells. The consequences of such quick ROS activation on cell-damage and inflammatory response are presently under investigation.
Candida albicans biofilm development in catheter-associated urinary tract infections (CAUTIs)

Shane Cristy¹,², Jennifer Walker¹,²,³, Michael Lorenz¹,²

¹Dept. of Microbiology and Molecular Genetics, The University of Texas Health Science Center at Houston, TX, USA. ²Dept. of Microbiology and Infectious Diseases, MD Anderson UTHealth Graduate School of Biomedical Sciences, Houston, TX, USA. ³Dept. of Epidemiology, Human Genetics, and Environmental Sciences (EGHES), School of Public Health, University of Texas Health Science Center at Houston, TX, USA

Abstract

Candida albicans is the second most common species isolated from CAUTI biofilms. Severe CAUTI biofilms occlude the catheter and serve as a reservoir for pathogens that promote symptomatic infection. C. albicans biofilms have been extensively studied in oral, bloodstream, and in vitro models, identifying a complex transcriptional regulatory network. Despite the frequency CAUTIs, little is known about the molecular basis of C. albicans biofilm formation in the urinary environment. The objective of this project is to determine molecular mechanisms intrinsic to C. albicans that promote biofilm growth in CAUTIs. First, we measured biomass and structure in vitro of mutants lacking each of the major biofilm transcriptional regulators. We observed that mutants lacking ROB1 and TEC1 had substantially different phenotypes in urine media compared to growth in RPMI medium, suggesting there are niche specific adaptations in the regulatory network. We then used RNA-seq on biofilms to gain an unbiased view of the total transcriptome in SC5314 and two C. albicans CAUTI clinical isolates. These data show that urinary biofilm expression profiles share significant similarities to Spider-grown biofilms, but also many differences, reinforcing the niche specificity of these structures. We are testing both regulatory and effector mutants in a murine model that replicates clinical biofilms and accounts for the complexities of multispecies biofilms, the presence of host factors and immunity, and continuous nutrient exchange. The completion of this study should elucidate the major determinants of C. albicans biofilm development in urinary catheters, thus identifying potential targets for anti-biofilm drugs or catheter coatings.
New Generation antifungals Developed Using Efflux Resistance Breaker (ERB) Technology

Charlotte Hind¹, Yiyuan Chen², Md Ashiqul Islam², Mahnoor Hassan², Barry Panaretou², J. Mark Sutton¹, Khondaker Miraz Rahman²

¹UKHSA, United Kingdom. ²King’s College London, United Kingdom

Abstract

Introduction: We have developed a novel approach to reduce efflux liability in antibiotic and antifungal classes, based on advanced structural modelling, identification of “efflux-resistance breaker” pharmacophores and detailed understanding of efflux liability in multidrug resistant (MDR) pathogens. This technology has been applied to the azole class of antifungals.

Methods: Computational tools including Biovia Discovery Studio and Amber v16.0 were used to study the interaction of azoles with MDR1 and CDR1 efflux pumps and identify suitable ERB fragments to direct ERB-azoles to the target residues. Fragments were linked to a modified azole pharmacophore using solution phase chemistry. Minimum inhibitory concentration50 (MIC50) values were determined using CLSI methods. In vitro DMPK, IV and oral maximum tolerated dose and pharmacokinetic studies were performed by the NIAID pre-clinical services. In vivo efficacy was demonstrated in a C.auris Galleria mellonella model and in a C.albicans mouse septicaemia model.

Results: Lead ERB-azoles demonstrated good activity against fungal pathogens including C.auris (MIC50 ≤0.03-2mg/L). Modified azoles without the ERB fragment were inactive against the target pathogens. ERB-azoles did not show any toxicity at 400mg/Kg in mice and demonstrated in vivo efficacy in a systemic lung infection model of C.albicans. The lead compound also showed statistically significantly better activity in a G.mellonella model of C.auris infection compared to fluconazole. The off-target toxicity screen, Safetyscreen44, did not reveal any issues.

Conclusion: We have observed excellent activity for ERB-azoles in MDR species, including in C.auris strains which have azole resistance through both up-regulation of multiple efflux pumps and ERG11 target mutations.
A087

Transcription factor Adr1 and its role in citrate utilization and gluconeogenesis in *C. albicans*.

Amelia White, Aaron Mitchell

University of Georgia, USA

Abstract

Opportunistic fungal pathogen *Candida albicans* often encounters host niches where it must survive on alternative carbon sources alone. In fact, alternate carbon sources are utilized during host cell invasion. In *Saccharomyces cerevisiae*, transcription factor Adr1 activates genes required for acetate and glycerol utilization. However, the Lorenz lab found some time ago that *ADR1* deletion mutants of *C. albicans* can grow on acetate and glycerol. Through preliminary screening and further phenotypic assays we have found that Adr1 is required for growth on citrate and malate, two intermediates of the TCA cycle. This phenotype is manifested in among multiple strains isolated from all known host niches. RNA sequencing and Nanostring comparisons of *adr1Δ/Δ* and wild-type strains show that Adr1 is required for expression of key TCA cycle and gluconeogenic genes. One Adr1-dependent gene, *MDH1*, encodes mitochondrial malate dehydrogenase. Both *mdh1Δ/Δ* and *adr1Δ/Δ* mutants are defective for growth on citrate. However, *mdh1Δ/Δ* mutants are able to grow on malate, suggesting that there may be a compensatory mechanism. Further investigation of *C. albicans* Adr1 will allow provide a better understanding of alternative carbon metabolism and its contribution to pathogenicity.
Global Translational Response of *Candida albicans* to Fluconazole

Saket Choudhary¹², Vasanthakrishna Mundodi³, Andrew D. Smith¹, David Kadosh³

¹Quantitative and Computational Biology, University of Southern California, Los Angeles, CA, USA. ²Present address: New York Genome Center, New York, NY, USA. ³Department of Microbiology, Immunology & Molecular Genetics, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Abstract

While transcriptional mechanisms that control the ability of human fungal pathogens to respond to treatment with antifungals have been well studied, considerably less is known about translational mechanisms. In order to gain a better understanding, we used ribosome profiling, a powerful genome-wide approach, to report the first global translational profile of a human fungal pathogen, *Candida albicans*, to treatment with the commonly used antifungal fluconazole. We identified 181 genes showing significantly increased and 147 genes showing significantly reduced translational efficiency (TE) in response to fluconazole treatment. A gene ontology (GO) analysis indicated that gene categories associated with the plasma membrane, cell cycle, mitochondrial envelope, transport as well as DNA-binding and oxidoreductase activities were strongly represented among the set of genes with increased TE. Interestingly, several genes important for DNA repair, cell wall biosynthesis, stress responses and signaling pathways (including the calcineurin pathway) as well as the SUT1 transcriptional regulator, involved in sterol uptake, showed increased TE in response to fluconazole. Gene categories associated with DNA- and RNA-binding proteins, DNA metabolic processes and protein synthesis showed significantly reduced TE upon fluconazole treatment. While we observed both similarities and differences among gene categories under translational vs. transcriptional control in response to fluconazole, there was very little overlap between individual genes showing altered TE vs. RNA differential expression (DE). Our findings suggest that *C. albicans* possesses specific translational mechanisms, distinct from transcriptional mechanisms, that are important for responding to fluconazole and other stress conditions, which could potentially be targeted by novel antifungal therapeutics.
The unintended consequences of antifungal treatment

Gabriela Ribeiro, Logan Tomlinson, Delma Childers

Aberdeen Fungal Group at University of Aberdeen, United Kingdom

Abstract

_Candida albicans_ is the leading cause of invasive candidiasis, but in recent years antifungal-resistant _Candida_ species have emerged as a serious clinical concern. _C. glabrata_ is the second leading cause of invasive candidiasis in much of North America and Europe and a subset of clinical isolates have intrinsic resistance to azole antifungals. While key resistance mechanisms to azoles and polyenes have been described, we have a poor understanding of the early physiological adaptations induced by antifungal exposure and how these adaptations affect pathogenesis and the emergence of resistance. To address this gap, we reviewed previously published ‘omics data for _C. glabrata_ and _C. albicans_ to gain insight into common and differing responses to antifungal insults. Most studies focused on few clinical antifungals and, despite differences in time points, drug concentrations, and growth medium, highlighted significant changes in carbon and lipid metabolism, translation, cell wall organization, and transporter gene/protein expression. Changes in metabolism, translation, and the cell wall can significantly affect yeast fitness in vivo. Therefore, we are also investigating how in vitro antifungal exposure affects cell wall composition, subsequent innate immune interactions and host survival in a waxworm infection model. By determining the broader adaptive responses of pathogens to antifungal treatment, we will provide a better understanding of the unintended consequences of therapy and how these shape infection outcomes.
Uncovering the role of potassium transport in C. auris skin niche biofilm growth

Emily Eix, Chad Johnson, Kayla Wartman, Grace Multhauf, Dhanansayan Shanmuganayagam, Angela Gibson, Jeniel Nett
University of Wisconsin-Madison, USA

Abstract

Background: Candida auris poses an urgent public health threat due to its propensity to colonize human skin, leading to rapid nosocomial spread. It is critical to understand how C. auris grows on skin to prevent skin colonization in healthcare settings.

Methods: We used synthetic sweat medium and ex vivo porcine skin to study C. auris skin colonization. We systematically removed individual components of the sweat medium and assessed biofilm growth in vitro in synthetic sweat as well as colonization of skin ex vivo. Additionally, we disrupted genes involved in potassium transport via homologous recombination and assessed biofilm growth and skin colonization.

Results: We show that potassium is necessary for C. auris biofilm growth in synthetic sweat medium. When potassium bicarbonate is removed from synthetic sweat, C. auris no longer grows as a biofilm. Multiple potassium sources restore the growth of C. auris when supplemented to sweat medium lacking potassium bicarbonate. We found that the deletion of the potassium transporter gene TRK1 results in a biofilm growth defect in synthetic sweat as well as a skin colonization defect using ex vivo models, which can be restored with the addition of large amounts of potassium.

Conclusions: These studies reveal the importance of potassium uptake in C. auris skin colonization and biofilm growth. As potassium transport in C. auris is not well-understood, further exploration of the mechanisms of potassium transport and its influence on biofilm growth provides new insight and points toward potential treatments for this deadly pathogen.
The two sides of Candida albicans cell type regulator Wor3

Katharina Goerlich, Aaron Mitchell

University of Georgia, USA

Abstract

In the fungal pathogen Candida albicans, biofilm formation depends upon the yeast-to-hyphal transition. Hyphae express virulence functions that include surface adhesins that ensure biofilm integrity. Previous studies have defined six transcription factors that are considered master transcriptional regulators of biofilm formation. One of the master regulators is Efg1. Efg1 has been extensively characterized in the type strain of C. albicans, SC5314, and its marked derivatives. Our recent studies defined a principal set of Efg1-responsive genes whose expression is significantly altered by an efg1ΔΔ mutation across 17 clinical isolates. Principal targets comprise fewer than 20% of the Efg1 targets in strain SC5314. Surprisingly, the principal targets include an Efg1 activated gene, WOR3, that was known to function in the Efg1-repressed mating pathway. WOR3 specifies a transcription factor, and has been characterized only under non-biofilm growth conditions. Previous research on WOR3 shows that it is associated with regulation of white-opaque switching, where it ultimately antagonizes Efg1. Past studies have also shown that Wor3 acts in parallel with Efg1 to inhibit GI tract colonization. We find that WOR3 promotes biofilm formation, based on analysis of wor3ΔΔ null mutants and WOR3-overexpression strains. Nanostring profiling indicates that Wor3 activates expression of many Efg1 principal targets. The sharing of target genes argues Wor3 is integrated into the Efg1 regulatory network. With engineered mutants in diverse C. albicans strains we aim to explore this newly found function of Wor3 in the biofilm regulatory network.
Candida albicans intestinal carriage in healthy volunteers: interactions with the microbiota, diet, host genetics and immune response

Margot Delavy\textsuperscript{1}, Natacha Sertour\textsuperscript{1}, Etienne Patin\textsuperscript{2}, Emmanuelle Le Chatelier\textsuperscript{3}, Florian Dubois\textsuperscript{4}, Violaine Saint-André\textsuperscript{5}, Lluis Quintana-Murci\textsuperscript{2}, Darragh Duffy\textsuperscript{4}, Christophe D'Enfert\textsuperscript{1}, Marie-Elisabeth Bougnoux\textsuperscript{1,6}

\textsuperscript{1}Institut Pasteur, Université Paris Cité, INRAE USC2019, Fungal Biology and Pathogenicity Unit, France. \textsuperscript{2}Institut Pasteur, Université Paris Cité, CNRS UMR2000, Human Evolutionary Genetics Unit, France. \textsuperscript{3}INRA, MetaGenoPolis, Jouy-en-Josas, France. \textsuperscript{4}Institut Pasteur, Université Paris Cité, Translational Immunology Unit, France. \textsuperscript{5}Institut Pasteur, Université Paris Cité, Bioinformatics and Biostatistics Hub, France. \textsuperscript{6}Assistance Publique des Hôpitaux de Paris, Hôpital Necker-Enfants-Malades, Service de Microbiologie Clinique, Unité de Parasitologie-Mycologie, France

Abstract

Candida albicans is a commensal yeast present in the gut of most healthy individuals but with highly variable concentrations. However, little is known on the interaction between the host factors and the extend of such concentrations.

We investigated how the microbiota, and the host’s diet and genetics modulated C. albicans carriage, in 695 healthy individuals from the Milieu Intérieur cohort. C. albicans intestinal carriage was detected in 82.9\% of the subjects by quantitative PCR. Using linear mixed models and multiway-ANOVA, we explored C. albicans intestinal levels with regards to the subjects’ gut microbiota composition and diet. We thus showed that a SCFA-producer bacteria, was the only species whose relative abundance was negatively correlated with C. albicans concentrations. Diet contributed to C. albicans growth, with subjects’ salt consumption and snacking habits being associated to higher C. albicans carriages. Also, by Genome-Wide Association Study, we highlighted 26 SNPs associated to C. albicans colonization.

In addition, we found that C. albicans intestinal levels influenced the host immune response. We analyzed the transcription levels of 546 immune genes and the concentration of 13 cytokines in the subjects’ blood, after stimulation with C. albicans cells and showed positive associations between the extent of C. albicans intestinal levels and NLRP3 expression, and IL-2 and CXCL5 concentrations.

The relative importance of the associations that we identified are still to be determined. Nevertheless, these finding possibly open the way for new intervention strategies to curb C. albicans intestinal overgrowth.
Conflict or Cooperation? A Role for Copper in *Candida-Staphylococcus* Biofilms

Seána Duggan

MRC CMM University of Exeter, United Kingdom

Abstract

Co-infections caused by the fungus *Candida albicans* and the bacterium *Staphylococcus aureus* result in prolonged treatment and poorer patient outcome due to a phenomenon termed “lethal synergy” where *C. albicans* and *S. aureus* interact to enhance virulence resulting in worse disease. Dual species biofilms also exhibit enhanced biomass and antimicrobial resistance compared to their single species counterparts. Mono and dual species *C. albicans* and *S. aureus* were cultured to early and mature biofilms. Mature dual species biofilms demonstrated increased biomass and metabolic activity compared to their single species counterparts. To investigate the proteins driving this synergy, total protein was extracted from mature biofilms and subjected to tandem mass tagging and liquid chromatography coupled to mass spectrometry. The resulting proteomes revealed 261 proteins differentially expressed in the dual species biofilm. Notably, *C. albicans* expresses proteins involved in copper uptake (Ctr1, Mac1) while *S. aureus* decreases expression of a copper senser (CsoR), chaperone (CopZ) and transporter (Ctr1). Dual species biofilms cultured in copper deplete and replete conditions showed reduced synergistic phenotypes of biomass and metabolic activity, while their single species counterparts were unaffected. Furthermore, environmental copper status impacts the structure of the dual species biofilm as imaged by scanning electron microscopy. Together these data reveal copper as an important resource for *C. albicans* dual species biofilms and demonstrate a role for copper interplay in dual species synergy. This knowledge has the potential to unveil avenues to rationally interfere with synergism in co-infections.
Using intravital imaging in zebrafish to understand signaling underlying neutrophil-mediated immunity to C. albicans infection.

Nnamdi Baker
University of Maine, USA

Abstract

Candida albicans is a commensal fungus affecting immunocompromised patients due to their impaired innate immune response which is integral in preventing lethal invasive candidiasis. Neutrophils maintain immunity by being recruited to the infection site and clearing it through phagocytosis or production of extracellular traps. However, defects in recruitment lead to human disorders like WAS (Wiskott-Aldrich Syndrome), LAD (Leukocyte Adhesion Deficiency) or WHIM (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis) which all promote increased susceptibility to recurrent infection. Although we understand the molecular defects of each disease, it is unclear how those defects translate to altered phagocyte recruitment, phagocytosis, and fungal killing. Intravital imaging of mutant neutrophils in the context of infection could shed some light into how each defect affects distinct aspects of the neutrophil’s functional response. To quantify defects in neutrophil recruitment and clearance, we have monitored neutrophil recruitment in larval zebrafish during hindbrain injection of C. albicans. This route of infection models a systemic infection. Our preliminary results modeling loss of gradient sensing indicate that the CXCR2 receptor is important for immunity in this infection route, as expected. However, blockade of this receptor does not significantly diminish neutrophil recruitment to the infection site, suggesting that other functions of CXCR2 signaling are important for controlling candidemia. Future work will continue to examine these neutrophil immune pathways in control of wildtype as well as evasion-deficient strains of C. albicans. A cellular understanding of the roles of these pathways in candidiasis may lead to targeted treatments for increasing survival in immunosuppressed patients.
IntraORF-targeted gene editing in *Candida auris*

Robert Zarnowski\(^{1,2}\), Justin Massey\(^{1,2}\), Natthapon Soisangwan\(^{3}\), Anna Selmecki\(^{3}\), David Andes\(^{1,2}\)

\(^1\)Department of Medicine, University of Wisconsin-Madison, USA. \(^2\)Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, USA. \(^3\)Department of Microbiology and Immunology, University of Minnesota, USA

**Abstract**

**BACKGROUND:** Emergent *Candida auris* is easily transmissible and frequently forms biofilms resistant to antifungal drugs and the host response. Due to its recent emergence, the basic principles of *C. auris* pathogenesis remain poorly understood. Of central importance to the study of this organism is the ability to generate null mutants for analysis in pathogenesis models. The *C. auris* genome is characterized by heterogeneity and frequent short UTR repeat sequences surrounding genes. Thus, existing homologous recombination-based gene manipulation strategies in *C. auris* often yield undesirable misintegration of the deletion cassette. Here we present an alternative approach that achieves efficient homologous recombination for gene editing.

**METHODS:** We constructed series of gene deletion cassettes that targeted distinct sequences within both flanking UTRs and ORFs of genes of interest in four distinct *C. auris* clades. We complemented the deletion mutants utilizing a novel codon-optimized zeocin resistance gene marker.

**RESULTS:** We initially targeted ADE2 and observed correct genome integration greater for gene deletion cassettes homologous to intraORF sequences than for those targeting respective UTRs (transformation success rate >15% vs <1%, respectively). The integration was similarly successful in all four tested *C. auris* clades. We validated this approach for six previously uncharacterized *C. auris* MNT mannosyltransferases, which were successfully deleted and complemented using this intraORF-targeting approach.

**CONCLUSION:** We find targeting intraORF sequences of genes of interest permits rapid generation of null and complement mutants in *C. auris.*
Mutations in sphingolipid biosynthesis gene FEN1 arise during the evolution of echinocandin resistance in gut-colonizing Candida glabrata

Dr. Yasmine Hassoun, Dr. Ariel Aptekmann, Rosa Hernandez, Christopher Quinteros, Dr. Erika Shor, Dr. David Perlin

Center For Discovery and Innovation, USA

Abstract

Due to high rates of azole resistance in non-albicans Candida species such as C. glabrata, echinocandins are now recommended as the first-line antifungal treatment. However, echinocandin resistance in C. glabrata has been increasing. Echinocandins target fungal cell wall biosynthesis by inhibiting 1,3-β-glucan synthase (GS) encoded by FKS1 and FKS2 genes. We have shown that the gut is an important reservoir for the development of C. glabrata echinocandin resistance. To investigate more closely the development of resistance to the echinocandin caspofungin, we used a mouse model of gastrointestinal (GI) C. glabrata colonization. Gut-colonizing C. glabrata strains that had evolved reduced caspofungin susceptibility in vivo (fungal rebound during treatment) and in vitro (elevated MIC) but lacking fks mutations were analyzed by whole genome sequencing. This analysis identified multiple strains with mutations in FEN1, which encodes a very long chain fatty acid synthase essential for sphingolipid biosynthesis. In vivo competition experiments showed that fen1 mutants show reduced fitness relative to WT cells in the absence of caspofungin but increased fitness in its presence. Moreover, we identified several different clinical FEN1 mutations, which we then showed to phenocopy the fen1∆ mutant, causing reduced susceptibility to caspofungin. Lipidomic analysis showed that FEN1 mutants had increased levels of phytosphingosine (PHS), which was previously implicated in desensitizing GS to echinocandin inhibition. Interestingly, deletion of YPC1, the ceramidase responsible for PHS synthesis, sensitized C. glabrata to caspofungin and was epistatic to fen1∆. These results highlight the importance of sphingolipid biosynthesis in the emergence of echinocandin resistance in vivo.
Identifying and characterizing antifungals with novel activity against the emerging fungal pathogen Candida auris

Lina Lim¹, John A. Porco², Lauren E. Brown², Nicole Robbins¹, Leah E. Cowen¹

¹University of Toronto, Canada. ²Boston University, USA

Abstract

Billions of people are infected by fungal pathogens every year, resulting in at least 1.5 million deaths worldwide. An emerging pathogenic yeast, Candida auris, causes invasive candidiasis with a mortality rate of 29-53%. Treatment is complicated by the emergence of drug-resistant isolates, with 87-100% of clinical strains resistant to the most widely-deployed antifungal drug class, the azoles. The aim of the study is to identify and characterize molecules with novel antifungal activity against C. auris. The Boston University Center for Molecular Discovery (BU-CMD) library, consisting of 3,936 chemically-diverse compounds, was screened against an azole-resistant C. auris strain to identify both single agents and fluconazole potentiators. The screen identified 44 compounds, of which five were prioritized based on novelty and potency. The spectrum of activity of the compounds was assessed by standard dose-response assays against different C. auris clades, as well as against other evolutionary-diverse fungal pathogens including Candida albicans, Cryptococcus neoformans, and Aspergillus fumigatus. To identify compound mechanism of action, future work will employ haploinsufficiency profiling (HIP) to identify heterozygous deletion mutants hypersensitive to compound treatment. Resistant mutants can also be selected for and mutations that confer resistance to hit molecules can be identified by whole genome sequencing (WGS). Finally, the therapeutic potential of the target compounds can also be examined by co-culturing human cells with C. auris to assess toxicity to mammalian cell lines and specificity to fungal cells. Overall, this project has the potential to identify novel therapeutics to treat C. auris infections.
Elucidating the role of mitochondria in the Candida glabrata response to echinocandins

Irene Gonzalez-Jimenez¹, Rocio Garcia-Rubio¹, Yasmine Hassoun¹, Christopher Quinteros¹, Erika Shor¹,², David S Perlin¹,²,³

¹Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, New Jersey, USA. ²Department of Medical Sciences, Hackensack Meridian Health, Nutley, New Jersey Institute of Medicine, USA. ³Lombardi Comprehensive Cancer Center, Department of Microbiology and Immunology, Georgetown University, Washington, DC, USA

Abstract

In recent years, the increasing emergence of echinocandin resistant isolates of Candida glabrata is limiting therapeutic options for patients infected by this species. Clinical echinocandin resistance is associated with mutations in FKS1 and FKS2 genes, which encode echinocandin target, B-1,3-glucan synthase (GS), an enzyme involved in the synthesis and maintenance of the cell wall. Previous studies have shown that resistance to another drug class, the azoles, can be caused by mitochondrial defects in C. glabrata, but whether mitochondria play a role in echinocandin resistance is unknown. Interestingly, several published studies have reported that in Saccharomyces cerevisiae Fks1 co-purifies with mitochondria, and we recently found that in C. glabrata Fks1 coimmunoprecipitates with mitochondrial proteins. We have also shown that echinocandin treatment of C. glabrata induces the production of reactive oxygen species (ROS). However, we found that this ROS production is largely unaffected in mutants lacking components of the mitochondrial respiratory chain, suggesting that echinocandin-induced ROS have predominantly non-mitochondrial origins. Our current studies are focusing on investigating the GS-mitochondrial interaction using imaging methods to help elucidate the role of mitochondrial dynamics in the GS-echinocandin interaction. We are also working on identifying the molecular nature of ROS production in echinocandin-treated C. glabrata using electron spin resonance techniques in order to, ultimately, pinpoint its sources. Together, these studies will help understand the roles of mitochondria and ROS in the C. glabrata response to echinocandins and echinocandin resistance.
Defining essential roles of calcineurin in *Candida glabrata*

Matthew Pavesic, Andrew Gale, Kyle Cunningham

Johns Hopkins University, USA

Abstract

Calcineurin signaling is required for *Candida glabrata* proliferation in mice, while its main downstream effector (Crz1) is not. We screened for *in vitro* genetic conditions that similarly required calcineurin for proliferation using a transposon insertion mutagenesis approach. Out of this screen, 50 non-essential genes became essential for cultivation in the calcineurin-inhibitor FK506. Several genes involved in cell wall biogenesis (FKS1, DCW1, FLC1) have essential paralogs whose expression is induced by calcineurin and Crz1 signaling. Micafungin treatment, genetic deletion of FKS1, DCW1, or FLC1, or exogenous stress also induced calcineurin activation and the expression of these paralogs. This finding suggests calcineurin regulates a set of back-up enzymes that compensate for cell wall damage. Disruption of genes involved in vesicular trafficking (especially the AP-1 and ESCRT complexes) produced strong dependence on calcineurin in growth assays. Transposon disruption of N-linked glycosylation and GPI-anchor biosynthesis in the endoplasmic reticulum also sensitized *C. glabrata* cells to FK506. We validated these findings using both knockout mutants and inhibitors such as tunicamycin and manogepix. Interestingly, these drugs were fungistatic to wild-type and *crz1Δ* mutants of *C. glabrata* but became fungicidal when calcineurin is lost. Thus, during ER stress, calcineurin promoted cell survival independent of Crz1, similar to the known role of calcineurin during invasive candidiasis. By further exploring the mechanism through which calcineurin promotes cell survival *in vitro* we may obtain a better understanding of how it does so *in vivo* and gain new insights into combating *C. glabrata* infections.
Complex Transcriptional Networks Control Morphogenetic Switching in the Human Fungal Pathogen *Candida auris*

Phan-Canh Trinh\(^1\), Sabrina Jenu\(^1\), Hossein Arzani\(^1\), Tamires Bitencourt\(^1\), Philipp Penninger\(^1\), Anuradha Chowdhary\(^2\), Karl Kuchler\(^1\)

\(^1\)Medical University of Vienna, Max Perutz Labs Vienna, Campus Vienna Biocenter, A-1030 Vienna, Austria. \(^2\)Medical Mycology Unit, Department of Microbiology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India

**Abstract**

*Candida auris* is a newly emerging opportunistic human fungal pathogen, causing serious hospital outbreaks of infections with high mortalities in immunocompromised individuals. The pronounced skin tropism, its pan-antifungal multidrug resistance traits (MDR), and the ease of transmission in clinical settings prompted the WHO to put *C. auris* on center stage as a top 3 fungal priority pathogen for drug discovery. Interestingly, morphogenetic switching between *White* and *Brown* morphologies as well as filamentation may control fungal fitness in the host. However, the contribution of morphogenesis to pathogenesis and virulence of *C. auris* remains enigmatic. Here, we show that morphogenetic switching from *White* to *Brown* cells engages transcriptional changes related to adhesion, filamentation, nutrient-sensing, skin adhesion and chromatin modifications. By comparing transcriptional profiles of stable *White* and *Brown* cells emerging from a pan-antifungal multidrug-resistant clinical isolate, we identify 285 differentially expressed genes, out of which at least 28 transcription factors are implicated in the regulation of filamentation, biofilm formation, interspecies interaction, as well as nutrient-sensing and metabolism. Remarkably, the systematic phenotyping of *White* and *Brown* cells reveals that adhesion, metabolism, and cell wall architecture correlate with altered transcriptomes and adaptation to different host tissues such as skin. Most importantly, we dissect the underlying transcriptional regulatory networks that define cell fate switches in *C. auris*. In summary, the integrative approach of RNA-seq and reverse-genetic manipulations demonstrate that *C. auris* can exploit two distinct morphogenetic cell types to control skin adhesion, fitness in the host as well as immune evasion.
B103

Essential TORC1 components required in *Candida albicans* cell wall stress endurance

Maikel Acosta-Zaldivar1,2, Wanjun Qi1,2, Caitlin Syphurs1,2, Sanya Thomas1,2, Biju Issac1, Gary P. Moran3, Liang Sun1, Joann Arce1,2, Julia R. Kohler1,2

1Boston Children's Hospital, USA. 2Harvard Medical School, USA. 3Trinity College Dublin, Ireland

Abstract

The Target of Rapamycin Complex 1 (TORC1) is a major regulator of growth-versus-stress responses in eukaryotic cells. We previously showed that truncating *C. albicans* Tor1 kinase for the domain that is least conserved with its human ortholog, induces severe hypersensitivity to cell wall stress. This region comprises protein-protein interaction domains, HEAT repeats, that physically contact the essential TORC1 scaffold and regulator Kog1. Phosphorylation of MAP kinases Mkc1 and Cek1 was aberrant in Tor1Del381 cells, indicating defective cross-signaling with cell wall stress pathways. Surprisingly, cell wall stress-responsive genes were upregulated in cells exposed to superoxide stress, with Tor1Del381 cells exhibiting aberrant responses. We questioned 1. whether cell wall stress hypersensitivity of cells lacking 8 N-terminal HEAT repeats (“Tor1Del381 cells”) is due to their simultaneous hyper- and hypoactive TORC1 signaling state, and 2. whether their cell wall stress phenotypes are due to a defective physical interaction with Kog1. We engineered constitutively hypoactive Tor1L1414P and partially hyperactive Tor1E2368K mutants and found that baseline low TORC1 activity increased cell wall stress endurance. Cells slightly repressing both *TOR1Del381* and *KOG1* from tetO, showed severe growth defects and overly exuberant chitin cell wall deposition during micafungin exposure, compared with controls. In other respects, their phenotypes were not identical, suggesting that only specific defects in their cell wall stress responses might relate to defective interactions of mutant Tor1Del381 with Kog1. We propose that fungal-specific regions of conserved essential regulators in TORC1 might be suitable targets for small molecules that potentiate inhibitors of cell wall biosynthesis.
Tor1 kinase N-terminal HEAT repeats are required for *Candida albicans* oxidative stress responses and adequate HOG signaling.

Wanjun Qi¹,², Maikel Acosta-Zaldivar¹,², Caitlin Syphurs¹,², Sanya Thomas¹,², Biju Issac¹, Gary Moran³, Liang Sun¹, Joann Arce¹,², Julia Köhler¹,²

¹Boston Children’s Hospital, USA. ²Harvard Medical School, USA. ³Trinity College Dublin and Dublin Dental University Hospital, Ireland

Abstract

Target of Rapamycin Complex 1 (TORC1) makes essential decisions to direct cellular resources toward growth and proliferation in favorable conditions, or toward stress and survival responses in adverse environments. The least conserved region of fungal and human Tor kinases is the N-terminal HEAT domains, a large array of protein-protein interaction regions.

Cells that express a mutant *TOR1* allele from repressible *tetO*, that encodes a Tor1 protein lacking the 8 most N-terminal HEAT repeats (Tor1⁰Del381 cells) were hypersensitive to oxidative stress compared with cells expressing wild type *TOR1* from *tetO* or from the native promoter. Hog1 kinase was hypophosphorylated in Tor1⁰Del381 cells in response to superoxide stress, suggesting that loss of N-terminal HEAT repeats disturbed cross-signaling between the HOG and TORC1 systems. Combining transcriptional- with metabolomics analysis showed that Tor1⁰Del381 cells aberrantly regulated pathways important in oxidative stress, like the pentose phosphate shunt. As Tor1⁰Del381 cells showed features of hyper- as well as hypoactive TORC1 signaling, we engineered cells with hypoactive *TOR1¹L1414P* - and partially hyperactive *TOR1¹E2368K* mutations to distinguish the effect of the TORC1 activity state on oxidative stress management, from specific defects of Tor1⁰Del381 cells. Decreased baseline TORC1 activity of *TOR1¹L1414P*-expressing cells increased their tolerance of oxidative stress. Cells lacking wild type activity of the TORC1 regulator and scaffold Kog1, which contacts Tor1 N-terminal HEAT repeats, also showed altered sensitivity to different oxygen radicals, while maintaining appropriate translation regulation during superoxide stress. Specific non-conserved regions of essential, overall conserved regulators, might become targets of domain-based drug discovery.
Clinical isolates of *Candida auris* show elevated salt tolerance

Milena Kordalewska, Geselle Cancino-Prado, Kevin D. Guerrero, Tony N. Elias, David S. Perlin

HMH Center for Discovery and Innovation, USA

Abstract

Background

The threat of invasive fungal infections has been recently exacerbated by the emergence of *Candida auris*, which, in contrast to most human-pathogenic *Candida* species, is characterized by elevated tolerance to various stressors (salts, temperature, drugs). While important progress has been made in understanding of *C. auris* antifungal drug resistance, its salt tolerance received much less attention.

Methods

*C. auris* clinical isolates of all clades, CRISPR/Cas9-generated knockout strains of *HOG1* (mitogen-activated protein kinase), *ENA1* (potassium/sodium efflux P-type ATPase), and *NHA1* (sodium/hydrogen antiporter), and control strains of *C. glabrata*, *C. albicans*, and *C. parapsilosis* were studied. Cells were inoculated into salt-supplemented YPD (1-20% (w/v) NaCl, KCl, LiCl, or CaCl$_2$) and incubated with shaking at 37°C for 24 h. Then, direct aliquots and serial dilutions were plated, and CFUs counted to determine the number of surviving cells.

Results

*C. auris* cells (irrespective of clade) survived significantly better in NaCl, KCl, LiCl, or CaCl$_2$ salt-supplemented YPD than *C. albicans* or *C. glabrata* and exhibited salt tolerance levels comparable to *C. parapsilosis*. *C. auris ΔHOG1* transformants exhibited significantly lower survival in all salts. Moreover, sodium and lithium salt tolerance were decreased in Δ*ENA1* but not Δ*NHA1* knockout *C. auris* strains.

Conclusion

Hog1 plays a central role in salt stress tolerance in *C. auris*, while Ena1 is involved in sodium and lithium salt tolerance. Our discoveries lay the foundation for further studies to elucidate molecular mechanisms responsible for the elevated salt stress tolerance in *C. auris*. 

Deciphering the interdependent roles of SPE1 and SPE2 for polyamine homeostasis during Candida albicans growth and morphogenesis

Ruvini Pathirana\textsuperscript{1}, Carolina Herrera\textsuperscript{1}, Geysira Vela\textsuperscript{1}, David Kadosh\textsuperscript{2}

\textsuperscript{1}Department of Biology & Chemistry, Texas A&M International University, USA. \textsuperscript{2}Department of Microbiology, Immunology & Molecular Genetics, University of Texas Health Science Center at San Antonio, USA

Abstract

Polyamines are aliphatic polycations essential for cell growth and differentiation in all organisms. Putrescine, spermidine, and spermine are the three major polyamines found in fungi. The only known pathway for de novo polyamine synthesis in yeasts is through the decarboxylation of ornithine. Although the inhibitors of polyamine biosynthesis were identified as potential antifungal targets, their mechanisms in C. albicans physiology are largely unknown. To further explore the polyamine biosynthesis and its regulation within C. albicans, we knocked out two coregulatory genes in yeast polyamine biosynthesis pathway, SPE1 and SPE2 which have ornithine decarboxylase activity and S-adenosylmethionine decarboxylase activity, respectively. Interestingly, the requirement for spermidine for growth was much less in Δ/Δspe2 mutants and Δ/Δspe1 Δ/Δspe2 double mutants than in Δ/Δspe1 mutants. All mutant cells deprived of polyamines are unusually large due to expanded vacuoles likely indicating the enhanced polyamine transporter activity across the vacuolar membrane of the yeast cell. Deletion of SPE1 and SPE2 completely abolished filamentation in agreeing with previous studies (Naseem et al, 2019; Schrevens et al, 2018; Herrero et al, 1999). We, therefore, analyzed the concentration-dependent effects of polyamines. We found that the required polyamine quantity vastly varies among growth phenotypes and is partly due to pH changes. Currently, we are investigating whether the absolute requirement of polyamines or increased sensitivity to polyamine toxicity is responsible for the growth rate reduction of these mutant yeasts. These findings would facilitate unraveling the regulatory mechanisms of de novo polyamine biosynthesis and its pleiotropic effects in C. albicans growth and morphogenesis.
Dual RNAseq reveals aneuploidy-specific fungal-host interactions during oral infection

Hung-Ji Tsai¹, Norma Solis², Chris Seward³, Petra Vande Zande⁴, Anna Selmecki⁴, Scott S. Filler², Vinnie Bruno⁵, Anja Forche⁶

¹University of Birmingham, United Kingdom. ²Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, USA. ³Pacific Northwest Research Institute, USA. ⁴University of Minnesota, USA. ⁵University of Maryland, USA. ⁶Bowdoin College, USA

Abstract

The oral cavity is one of the few host niches in which C. albicans can grow as both a commensal and a pathogen. Oropharyngeal candidiasis (OPC) can develop as a consequence of acquired immunodeficiency, underlying diseases, and treatment with broad-spectrum antibiotics, corticosteroids or chemotherapy. We recently showed that when C. albicans proliferates in the oropharyngeal cavity during experimental OPC, it acquires large-scale ploidy changes with an over-representation of strains with whole chromosome (Chr) trisomies for Chr5 (AAB) or Chr6 (ABB and AAB). We hypothesized that these trisomies would affect survival in the host and assessed the virulence of representative strains during OPC. Surprisingly, all strains exhibited a commensal-like phenotype compared to the euploid progenitor. To understand how trisomies of Chr5 or Chr6 alter host-pathogen interactions, we performed RNA-seq on the euploid progenitor and strains trisomic for Chr5 (AAB) or Chr6 (ABB and AAB). We hypothesized that these trisomies would affect survival in the host and assessed the virulence of representative strains during OPC. Surprisingly, all strains exhibited a commensal-like phenotype compared to the euploid progenitor. To understand how trisomies of Chr5 or Chr6 alter host-pathogen interactions, we performed RNA-seq on the euploid progenitor and strains trisomic for Chr5 and Chr6 grown in vitro under in vivo-mimicking conditions. We also performed dual-species RNA-seq on tongue scraping samples from mice with OPC. We show that trisomies are maintained both in vitro and during infection. Preliminary RNAseq results suggest that the transcriptional profiles of the Chr6 trisomic strains are similar to each other in vitro but differ in vivo, indicating allele-specific responses to the host, and that the Chr5 trisomic strain has a unique profile. Preliminary upstream regulator analysis of the host transcriptome identified pathways that were uniquely activated or repressed in a fungal strain-specific manner. Additional data analysis is currently ongoing to further elucidate the intricate interplay between C. albicans and its murine host.
Programmed +1 ribosomal frameshifting and adaptive aneuploidy in *Candida glabrata*

Joshua Schultz, Kyle Cunningham
Johns Hopkins University, USA

Abstract

Previous work using Hermes transposon Insertion Profiling (HIP) has identified numerous kinetochore genes as essential in *Candida glabrata* but not in *Saccharomyces cerevisiae*. One possible explanation for this disparity is the interruption of *CgBIR1* (encoding an essential subunit of the chromosomal passenger complex [CPC]) with a programmed +1 ribosomal frameshift (+1PRF). Although absent in *ScBIR1*, it is conserved in dozens of other yeast species, suggesting it plays a beneficial role in the regulation of Bir1 expression. One such role could be adaptive aneuploidy, where *Cg* clonal populations may rapidly generate diversity (aneuploids) in response to adverse conditions. Though the slippery site sequence CUU-AGG-C is well-characterized in *Sc*, its modes of regulation are unknown. To help understand possible modes of regulation, we analyzed the genomes of *Cg* and related yeasts for additional genes containing +1PRFs. Remarkably, we find the identical +1PRF slippery site in four other genes conserved in most species. Two of them, *ATS1* and *ABP140*, encode enzymes that modify tRNAs at and near the wobble position respectively and therefore may modulate frameshifting efficiency through a complex feedback loop. We are testing this hypothesis directly by quantifying frameshifting efficiency in *abp140Δ, ats1Δ*, and various ΔPRF mutants. Additionally, we are testing whether environmental conditions/stress can alter frameshift efficiency and Bir1 expression. Furthermore, the impact of Bir1 expression on adaptive aneuploidy is being investigated in *Cg* using HIP and other assays. These experiments will shed light on mechanisms of adaptive aneuploidy and the development of antifungal resistance in diverse eukaryotic pathogens.
Applying TurboID in *Candida albicans* to elucidate how eisosomes regulate cell wall growth

Carla Lanze, James Konopka

Stony Brook University, USA

Abstract

The ability of *Candida albicans* to resist host-mediated stress allows it to infect numerous sites in the human body. Specialized subdomains of the fungal plasma membrane known as eisosomes are important mediators of stress resistance. Mutants lacking the major eisosome protein Sur7 display sites of aberrant cell wall formation. Upon further study, it was shown that sur7Δ displays mislocalized patches of PI(4,5)P₂ (PIP₂) that correspond to large invaginations of cell membrane. We hypothesize that Sur7 regulates PIP₂ levels in *C. albicans* by recruiting phosphatidylinositol phosphatases to eisosomes. To test this hypothesis, we deleted all of *C. albicans*’ phosphatidylinositol phosphatase genes (INP51, INP52, and INP54) and found that inp51Δinp52Δ and inp52Δinp54Δ mutants phenocopy features of sur7Δ such as increased sensitivity to cell wall stress, misregulated PIP₂, abnormal cell wall growth, and defective invasive growth. To further probe the relationship between eisosomes and the Inp proteins, we adapted a proximity labeling assay, TurboID, to function in *C. albicans*. We tagged two eisosome proteins, Lsp1 and Sur7, with a BirA variant, to promote biotinylation of nearby proteins. Mass spectrometry revealed that many of the major eisosome proteins were pulled down with streptavidin beads, additionally the PIP₂ phosphatase Inp52 was detected. These studies suggest that eisosomes have a direct role in regulating the PIP₂ phosphatases. Delineating the mechanisms by which eisosomes regulate PIP₂ will help define how this lipid controls cell signaling, morphogenesis, and virulence in *C. albicans*. Furthermore, TurboID can be utilized to further the understanding of *C. albicans* biology.
Microevolution of *Candida auris* upon anidulafungin exposure in patients and *in vitro* uncovers novel CRZ1 mutants underpinning drug resistance

Hugh Gifford¹, Sarah Murphy², Ian Leaves¹, Arnab Pradhan¹, Alistair Brown¹, Tihana Bicanic², Rhys Farrer¹

¹University of Exeter MRC Center for Medical Mycology, United Kingdom. ²St George’s University of London, United Kingdom

Abstract

Introduction: *Candida auris* is a WHO critical priority fungal pathogen. We aimed to discover genetic factors underpinning echinocandin resistance emergence.

Methods: Clinical isolates from two critically ill patients with echinocandin-refractory *Candida auris* candidaemia despite apparent drug susceptibility were included (n = 6, bloodstream; n = 1, bronchoalveolar lavage fluid), all of which had susceptible MIC (minimum inhibitory concentration, 0.125-0.25 μg/ml) to echinocandins. *In vitro* microevolution assays were performed over 7 days in anidulafungin-containing media (8μg/ml). Genomes of parent (clinical) and daughter (evolved) isolates were extracted and sequenced (on/off drug, in triplicate) with Illumina paired-end reads. Variants were identified and compared using GATK and custom scripts.

Results: All isolates were Clade I. Microevolved isolates revealed two distinct colonial phenotypes, rapidly-growing “big” colonies (anidulafungin resistant, MIC>256 μg/ml) and slower-growing, “small”, tolerant daughter colonies (MIC 0.25 μg/ml). Whilst there was no chromosomal aneuploidy, we found a novel segment of copy number variation in the region containing *MRR1a*, a transcriptional regulator of *MDR1*, in 10/42 parent and evolved isolates. Comparison of microevolved daughter isolates with parents demonstrated a significant preponderance of disruptive variants (stop codons) in calcineurin-activated transcription factor CRZ1 in “big” (resistant) isolates (8/17) and none in “small” isolates (0/18). Only two *FKS1* mutations were observed in evolved isolates (1 “big” and 1 “small”).

Discussion: Our study using clinical and *in vitro* microevolution demonstrates a likely role for CRZ1 in echinocandin tolerance and the genesis of resistance in *C. auris*, as previously demonstrated in *C. glabrata*. 
Investigation of fungal phase separation, transcription, and a global repressor

Maureen Dowell, Mae Staples, Corey Frazer, Richard Bennett
Brown University, USA

Abstract

*Candida albicans* undergoes epigenetic switching between multiple phenotypic states, including reversible switching between “white” and “opaque” states. This cell fate transition is regulated by a network of eight transcription factors (TFs). Seven of the eight TFs contain prion-like domains (PrLDs). We previously showed that these PrLDs enable white-opaque TFs to undergo liquid-liquid phase separation.

One of the white-opaque network TFs is Ssn6, which has been well studied due to its role as a conserved global co-repressor with its partner, Tup1. This is of interest as analysis of TF phase separation has previously focused on transcriptional activators. Here, we show purified Ssn6 is capable of undergoing phase separation with other *C. albicans* TFs in vitro. Moreover, Ssn6 drives exclusion of the RNA polymerase II C-terminal domain from condensates formed by these TFs, suggesting a mechanism for transcriptional inhibition. Ssn6 also forms liquid condensates in a U2OS cell model of phase separation, but the Ssn6 PrLDs are dispensable for formation of these condensates. Deletion of PrLDs also has only a modest effect on cell state switching. Interestingly, deletion of the disordered (but not prion-like) C-terminal domain of Ssn6 alters both U2OS condensate properties and frequency of white-opaque switching in *C. albicans*.

Current experiments are further examining how Ssn6 impacts the recruitment of RNA polymerase II to transcriptional condensates, as well as the role of the unstructured Ssn6 C-terminal domain in regulating cell state. We therefore plan to address the mechanism by which transcriptional repressors utilize phase separation to regulate gene expression.
Identification of small molecule inhibitors to *Candida albicans* phosphatidylserine synthase using a target-based screening

Yue (Aeric) Zhou\(^1\), Gregory Phelps\(^2\), Jeffrey Rybak\(^2\), Richard Lee\(^2\), Todd Reynolds\(^1\)

\(^1\)University of Tennessee Knoxville, USA. \(^2\)St. Jude Children's Research Hospital, USA

**Abstract**

Systemic infections by *Candida* spp. are associated with high mortality rates, partly due to the limitations in the current antifungals. Thus, there is a need for novel drugs. The membrane-bound phosphatidylserine synthase, Cho1, from *Candida albicans* is a potential drug target for new antifungals due to its importance in virulence, absence in the host and conservation among fungal pathogens. Inhibitors of Cho1 could serve as lead compounds for drug development, so we developed a target-based screen for inhibitors of Cho1. This enzyme condenses serine and cytidyldiphosphate-diacylglycerol (CDP-DAG) into phosphatidylserine and cytidyldiphosphate (CMP). Membrane-bound Cho1 was solubilized and purified using affinity chromatography coupled with size-exclusion chromatography (SEC). A nucleotidase-coupled malachite green-based assay that detects CMP released during catalysis was adapted for high-throughput screening. Approximately 7,900 molecules were interrogated in the primary screen from a set of curated repurposing libraries at a final concentration of 100 μM, followed by a counter-screening to eliminate potential false positives targeting the coupled nucleotidase. The overall screen has a promising average Z’ score of ~0.8, and 82 compounds with at least 60% activity inhibition to Cho1 were further evaluated in a dose-response assay. The IC\(_{50}\) values of the primary hits were calculated via dose-response curves and pan-assay interference (PAIN) compounds were disregarded. Finally, six non-PAIN compounds stood out with IC\(_{50}\) below 30 μM. Computational docking of these six compounds in the active site of Cho1 generated high scores. These compounds will be further characterized in live cells and can be potential leads for further drug development.
In vitro activities of amphotericin B, fluconazole, caspofungin, and micafungin against clinical isolates of *Candida auris* under both planktonic and biofilm-growing conditions

Hamid Badali\(^1\), Anupma Jyoti Kindo\(^2\), Nathan P. Wiederhold\(^3\), Alessandra Vaccaro\(^1\), Olabayo Ajetunmobi\(^1\), Jesus Vazquez Rodriguez\(^1\), Jose Lopez-Ribot\(^1\)

\(^1\)Department of Molecular Microbiology & Immunology, and South Texas Center for Emerging Infectious Diseases, The University of Texas at San Antonio, San Antonio, TX, USA. \(^2\)Department of Microbiology, Sri Ramachandra Medical College & Research Institute, SRIHER, Porur, Chennai, India. \(^3\)Fungus Testing Laboratory, The University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA

Abstract

Background: *Candida auris*, an emerging multidrug-resistant yeast, is a global public health concern as a causative of nosocomial outbreaks with high mortality among immunocompromised patients. The limited number of treatment options has raised major concerns in the management of *C. auris* infections. Therefore, we aimed to investigate the in vitro activities of commonly used antifungal drugs against *C. auris* isolates growing under both planktonic and biofilm conditions.

Methods: Forty *C. auris* isolates (confirmed by MALDI-TOF MS) were collected from blood cultures. Susceptibility testing against amphotericin B, fluconazole, caspofungin, and micafungin was determined using 96-well microdilution methods, according to the CLSI M27 for planktonic (PLK) growth, and an XTT-reduction assay for preformed biofilms (BF).

Results: Under planktonic conditions, 39 isolates showed high MICs for fluconazole (>32 µg/ml), while one strain had low MIC (2 µg/ml). Preformed biofilms of all 40 isolates were highly resistant to fluconazole (BF MICs from 128-512 µg/ml). Amphotericin B had low PLK MICs (0.063-0.5 µg/ml), but high BF MICs (0.5-32 µg/ml). A similar pattern was observed for the echinocandins, with relatively low PLK MIC for caspofungin and micafungin (0.25-1 µg/ml and 0.031-0.25 µg/ml, respectively), but somewhat elevated BF MICs (1->16 µg/ml and 0.25->16 µg/ml, respectively).

Conclusion: Overall, no pan-resistant strains were found in this collection according to PLK activity. However, BF MICs were significantly higher, which indicated their high levels of resistance against the antifungals tested. Further studies with a larger number of isolates from different clades are warranted.
Quinone reductases are important for *Candida albicans* resistance to oxidative damage and virulence

Rafael Gandra, James Konopka

Stony Brook University, USA

**Abstract**

*Candida albicans* is an effective pathogen because it can adapt to changing environmental conditions in the host, such as oxidative stress. Previous studies by our group identified a novel antioxidant pathway in *C. albicans* carried out by Flavodoxin-like proteins (FLPs), that localize to the eisosome domains of the plasma membrane and function as NAD(P)H:quinone reductases. Quinone reductases protect cells from oxidative damage by catalyzing a two-electron reduction of quinones, which is critical to avoid creating toxic semiquinone intermediates that lead to the formation of reactive oxygen species. FLPs promote resistance to a wide range of small quinones that are found in nature and are thought to reduce ubiquinone so that it can act as an antioxidant in the plasma membrane, and are essential for *C. albicans* virulence. We now investigate the role of Zta1, which has homology to mammalian ζ-crystallin proteins and is a member of a distinct family of quinone reductases named medium-chain dehydrogenase/reductases (MDR). Zta1 fused to GFP localized to the cytoplasm, not the eisosomes. Interestingly, Zta1-GFP was rapidly induced by quinones and other types of oxidants, consistent with a role for Zta1 in protecting against oxidative stress. Deletion of ZTA1 alone did not increase sensitivity to quinones. However, a quintuple mutant lacking ZTA1 and the FLP genes (zta1D pst1D pst2D pst3D ycp4D ) displayed increased sensitivity to 2-tert-Butyl-1,4-benzoquinone, indicating that these quinone reductases have some overlapping function. Current studies are defining the role of ZTA1 *C. albicans* virulence.
Biofilm formation in *Candida albicans* depends on prion-like domain residues in network transcription factors

Collin Ganser, Mae Staples, Richard Bennett

Brown University, USA

Abstract

*Candida albicans* is a leading cause of healthcare-acquired infections. This fungal species forms biofilms, which are controlled by a network of nine transcription factors (TFs) that regulate each other’s gene expression. The biofilm network resembles mammalian networks regulated by TFs acting at unusually large regulatory regions or ‘super-enhancers’. Several TFs that regulate mammalian super-enhancers are proposed to form condensates, partially due to weak multivalent interactions between TFs. In *C. albicans*, seven of the nine biofilm TFs possess prion-like domains (PrLDs), which enable multivalency and promote liquid-liquid phase separation. We hypothesized that *C. albicans* biofilm formation requires TF condensates to drive biofilm gene expression. To test this, we analyzed mutant TFs *in vitro* and by ectopic expression in mammalian cells to visualize condensate formation, as well as in *C. albicans* to measure biofilm formation. Four biofilm TF PrLDs promote condensate formation in mammalian nuclei. Purified TFs also form condensates *in vitro*, and these condensates can recruit the C-terminal domain (CTD) of RNA Polymerase II. PrLDs with key residues mutated (e.g., substitution of aromatic amino acids) were unable to form condensates or support biofilm formation. These results demonstrate that *C. albicans* requires TF PrLDs to form biomolecular condensates for their functionality. The ability of TF condensates to recruit RNA Polymerase II CTD suggests that this promotes the transcription of target genes. Ultimately, this study provides insights into the transcriptional regulation of biofilm formation in a clinically-relevant species and suggests that disruption of biomolecular condensates could be a target for therapeutic intervention.
B116

Functional genomic analysis of genes important for hyphal morphogenesis in the fungal pathogen *Candida albicans*

Aiman Farheen

University of Toronto, Canada

Abstract

Infectious fungal diseases impose a significant burden on human health, leading to ~1.5 million deaths per year. *Candida albicans* is a leading human fungal pathogen that is the causative agent of serious systemic infections, which are associated with mortality rates of ~40%. *C. albicans* can grow in yeast and filamentous forms, and the switch between these morphogenetic states is a key virulence trait. While the circuitry governing this transition has been studied, a comprehensive understanding of the genes involved in this process remains to be conducted. To characterize genes important for hyphal morphogenesis, we leveraged the Gene Replacement And Conditional Expression (GRACE) collection, a mutant library that was recently expanded to cover ~50% of the *C. albicans* genome. The GRACE library consists of strains where one allele of a gene is deleted, and the remaining allele is under the control of a doxycycline (DOX)-repressible promoter. To identify the *C. albicans* genes that orchestrate filamentation in host-relevant conditions, we performed a functional genomic screen with the expanded GRACE collection and identified 141 genes that were blocked in filamentation in RPMI supplemented with serum and incubated at 37 °C with 5% CO2, many of which were previously uncharacterized. Further investigation of the mechanisms by which prioritized genes affect filamentation is ongoing. This includes performing selection experiments to restore filamentation in non-filamentous mutants to elucidate mechanism via the identification of suppressor mutations. Ultimately, this project will identify mechanisms that enable *C. albicans* morphogenesis in host-relevant conditions to reveal key circuitry required for virulence.
Functional genomic analysis of *Candida albicans* protein kinases reveals modulators of morphogenesis in diverse environments

Saif Hossain, Yunjin Lee, Jessie MacAlpine, Nicole Robbins, Leah Cowen

University of Toronto, Canada

Abstract

Fungal diseases kill more than 1.5 million people globally each year, with the fungal pathogen *Candida albicans* being a leading cause of serious mycotic infection with unacceptably high mortality rates. The ability to transition between yeast and filamentous morphologies is a key virulence trait in *C. albicans* and cellular signaling cascades are integral in regulating morphogenesis in response to diverse inducing cues. However, a comprehensive assessment of how protein kinases regulate filamentous growth in response to diverse environments is lacking. In this study, we employed the GRACE (gene replacement and conditional expression) protein kinase library to identify *C. albicans* kinases that are important for morphogenesis. Through systematic phenotypic screens in six environmental conditions, we identified 25 negative regulators and 24 positive regulators of *C. albicans* filamentation. While some of these genes encode previously described morphogenetic regulators, many had yet to be implicated in this process. For example, the putative serine/threonine protein kinase orf19.3751 was identified as a repressor of filamentation. Further analyses suggested a role for this kinase as a cell cycle regulator. We also discovered a dual regulatory role for the kinases Ire1 and PKA in morphogenesis, specifically as negative regulators on solid medium but positive regulators in liquid medium. Follow-up investigations suggested that Ire1 modulates filamentation in part through the transcription factor Hac1 and in part through independent mechanisms. Collectively, our work provides novel insights into signaling that governs morphogenesis, an important virulence trait of a leading human fungal pathogen.
Overexpression of Genes Associated With Spaceflight Alters Traits Associated With Biofilm Formation

Evan Sidebotham, Michael Hawkes, Gabrielle Garlicki, Ian Cleary, Derek Thomas

Grand Valley State University, USA

Abstract

Candida infections are a frequent hospital acquired infection both in the US and worldwide. Such infections carry high morbidity and mortality rates and important economic repercussions. Another environment where C. albicans infection is of concern is in spaceflight. Microbial contamination is commonplace on the International Space Station Spaceflight and spaceflight reduces the human immune response. Since many humans are carriers of C. albicans there is therefore increased potential for it to cause an infection during spaceflight. C. albicans can grow in different morphologies which facilitates its ability to cause biofilms, three dimensional communities of microbial cells that form on surfaces. Biofilms appear to be important part of the ability to cause disease. Spaceflight alters the expression of numerous C. albicans genes and also leads to increased cellular aggregation, which may enhance biofilm formation. Here we demonstrate that increased expression of individual genes differentially regulated during spaceflight can alter biofilm formation and filamentation.
Conjugated action of Als3 and candidalysin for specific enterocytes targeting modulates intestinal response in fungal commensalism

Zi-Qi Gu, Kuan-Ting Lin, Yu-Huan Tsai

Institute of Microbiology and Immunology, National Yang Ming Chiao Tung University, Taiwan

Abstract

*Candida albicans* is the main fungal commensal in the gut, where it can translocate into circulation to cause systemic infection in immunocompromised. Despite of the importance of understanding the interaction between *C. albicans* and the gut, how *C. albicans* interacts with and even crosses the intestinal epithelium remains elusive. As compared to most of intestinal bacterial pathogens that mainly invade Peyer’s patches for successful infection, we found that these lymphoid tissues in the intestine are not targeted by *C. albicans*. We demonstrate that *C. albicans* mainly targets goblet cells and villous M cells in the intestinal villi. While the cadherin-interacting protein Als3 is not required for goblet cell targeting, both Als3 and candidalysin are required for villous M cell targeting. The villous M cell targeting results in EGFR activation and neutrophil infiltration. We further demonstrate that Als3 facilitates candidalysin-mediated c-Fos signaling and cytolysis in intestinal tissue culture dependent on EGFR signaling. Together, our findings demonstrate the cooperation of the two hypha-associated virulence factors, Als3 and candidalysin, in modulating intestinal inflammation via targeting specific enterocytes. These results highlight that *Candida* evolution toward reduced virulence factor expression may lead to stable gut colonization, and systemic infection in an immunocompromised host.
Exposure to agrochemicals induces resistance to azoles and tolerance to echinocandins in Candida auris.

Hao Li¹,², Yong-bing Cao³, Yuan-ying Jiang¹, Yang Feng¹

¹Department of Pharmacy, Shanghai Tenth People's Hospital, School of Medicine, Tongji University, Shanghai, China, China. ²Department of Physiology and Pharmacology, School of Basic Medicine and Clinical Pharmacy, China Pharmaceutical University, Nanjing, China, China. ³Department of Vascular Disease, Shanghai TCM-Integrated Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China, China

Abstract

Candida auris is an emerging global fungal public health threat. It is usually inherently resistant to fluconazole, and can rapidly develop resistance to other antifungal drugs. C. auris has been detected in different environmental habitats, and was found coexisting with agrochemicals on the surface of apples. But little is known about the effect of agrochemicals on the development of antifungal drug resistance in C. auris. Here we found exposure to tebuconazole caused cross-resistance to major triazole drugs including fluconazole, voriconazole and Posaconazole. Furthermore, some mutants were also tolerant to echinocandins including caspofungin and micafungin. Susceptibility to antifungals was measured by disk diffusion assays. Resistance was evidenced by reduced radius, and tolerance was evidenced by increased growth inside the zone of inhibition. We are performing whole genome sequencing to investigate the molecular mechanisms. Our study demonstrates environmental agrochemicals can induce multidrug resistance/tolerance in C. auris.
A Signature-tagged Conditional Mutant Strain Library for Functional Analyses of the *Candida auris* Kinome

Madiha BOUALI1, Colin CLAIRET2, Murielle CHAUVEL2, Imen FERJANI1, Hejer SOUGUIR1, Natacha SERTOUR2, David SHUM3, Changbin CHEN4, Christophe D’ENFERT2, Marie-Elisabeth BOUGNOUX2, Sadri ZNAIDI1

1Institut Pasteur de Tunis, Tunisia. 2Institut Pasteur, France. 3Institut Pasteur Korea, Korea, Republic of. 4Institut Pasteur of Shanghai, China

Abstract

*Candida auris* is an emerging multidrug-resistant and medical-care associated fungal pathogen. How this fungus expresses virulence traits and how it achieves high levels of drug resistance is unclear. Deciphering the underlying mechanisms can rely on the creation of genetic resources for functional analyses. We constructed a *C. auris* strain library for the systematic genetic perturbation of kinases, which are decision-making proteins and attractive targets for drug development. Mining of the *C. auris* genome allowed to identify 111 ORFs encoding putative kinases. Comparative analyses with the *S. cerevisiae* and *C. albicans* kinomes shows that the three species share similar proportions of kinase families, while around 15% of *C. auris* putative kinases display high sequence homology with *S. cerevisiae* and *C. albicans* essential kinases. We used a fusion-PCR strategy to create 111 signature-tagged (i.e. barcoded) cassettes for the replacement of each of the 111 putative kinase native promoters with a tetracycline-derivative repressible promoter in strain CBS12766. Initial functional validation assays with the resulting collection identified a subset of potential essential kinases (IPL1, CDC7, CDC28, RIO2) as well as kinases involved in cell-wall stress response (e.g. SLT2). Additional assays will exploit the presence of signature tags to simultaneously phenotype the mutant strains competitively grown in vitro and in vivo in an animal model of *C. auris* infection. This genetic resource will not only serve as a tool for illuminating pathways involved in the ability of *C. auris* to cause disease, but also implementing a drug discovery-oriented approach that targets *C. auris* kinases.
Differential sensing by the C. albicans Gpr1 receptor results in morphogenesis, β-glucan masking and survival in macrophages

Wouter Van Genechten, Stefanie Wijnants, Jolien Vreys, Patrick Van Dijck

KU Leuven, Belgium

Abstract

The human fungal pathogen, Candida albicans, is very proficient at several classical virulence factors such as morphogenesis, adhesion, biofilm formation and immune evasion through β-glucan masking. The protein kinase A (PKA) pathway is involved in both morphogenesis and β-glucan masking. Several signals converge onto the PKA pathway, but it contains only a single upstream G-protein coupled receptor, Gpr1. We identified specific residues within the N-terminal tail of Gpr1 that are required for methionine-induced morphogenesis through Tpk2. Furthermore, we observe that Gpr1-Gpa2 has an active role in exposing glucans. Even though Gpr1 is required for survival when C. albicans is challenged with macrophages, specifically disrupting morphogenesis did not attenuate this survival. Additionally, constitutive β-glucan masking did not improve C. albicans survival rates in the macrophage assay. Taken together, this indicates that Gpr1 may regulate additional mechanisms, possibly through glutamine 461, which are crucial in a macrophage context.
The transcription factor Rpn4 activates its own and efflux pumps’ expression to confer fluconazole resistance in Candida auris

Eve Chow¹, Yabin Song²,³, Xiaoli Xu¹, Jianbin Wang²,⁴, Jiaxin Gao¹,³, Yue Wang¹,⁵

¹A*STAR Infectious Diseases Labs, Agency of Science and Technology Research, Singapore. ²School of Life Sciences, Tsinghua University, China. ³State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, China. ⁴Beijing Frontier Research Center for Biological Structure, Tsinghua University, China. ⁵Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Abstract

Multi-drug resistance is an alarming feature of the emergent fungal pathogen Candida auris, with most clinical isolates resistant to the first-line antifungal drug fluconazole, causing difficult-to-treat infections with high mortality. However, the precise underlying mechanism is poorly understood. In this study, we conducted transposon-mediated genetic screens to profile genes whose inactivation causes fluconazole resistance in C. auris. We discover that mutation of genes encoding the Mub1/Ubr2 ubiquitin ligase complex results in high fluconazole resistance by stabilizing the short-lived transcription activator, Rpn4. Global transcriptomic analysis and combinatorial gene deletion reveal that Rpn4 causes fluconazole resistance by upregulating the expression of four efflux pump genes, SNQ21, SNQ22, MDR1, and CDR1, thereby increasing efflux activity of the cell. Rpn4 autoactivates its own expression by binding to a PACE element in its promoter, forming a positive autoregulatory loop. Rpn4 also promotes CDR1 expression, by binding to a PACE element in the CDR1 promoter. Furthermore, increased Rpn4 cellular levels cause cell aggregation and impair C. auris skin colonization in a mouse model. This study identifies Rpn4 as a key transcription factor that governs fluconazole resistance and influences morphogenesis and host skin colonization in C. auris.
Candida albicans lipases are fungal factors required for gut colonization and shaping the host microbiome.

Osama Elshafee¹, Lu Zhang², Sven Balluff³, Maria Joanna Niemiec⁴, Ketema Abdissa Merga⁴, Jakob L. Sprague¹, Lydia Kasper¹, Enrico Garbe⁵, Christine Beemelmanns³, Gianni Panagiotou², Ilse Jacobsen⁴, Sascha Brunke¹, Bernhard Hube¹

¹Department of Microbial Pathogenicity Mechanisms, Hans Knöll Institute, Jena, Germany. ²Department of Microbiome Dynamics, Hans Knöll Institute, Jena, Germany. ³Department of Anti-Infectives from Microbiota, Helmholtz-Institute for Pharmaceutical Research, Saarland, Germany. ⁴Department of Microbial Immunology, Hans Knöll Institute, Jena, Germany. ⁵Department of Host Fungal Interfaces, Hans Knöll Institute, Jena, Germany

Abstract

During gut colonization, Candida albicans constantly interacts with members of the host microbiome to compete for nutrients and space. These interactions determine the fungal ability to colonize the gut and potentially affects both microbiome composition and assembly. Yet, the fungal factors that facilitate gut colonization are still largely unknown. C. albicans possesses a large gene family of ten lipases, which are expected to have broad yet overlapping functions. We hypothesized that C. albicans lipases contribute to gut colonization and play a major role in microbial competition. To define the roles of C. albicans lipases in gut colonization, we developed a CRISPR-Cas9 system to create a library of lipase mutants, including mutants lacking the entire gene family (lip1-10Δ).

Our results show that C. albicans lipases enable the fungus to grow on a broad range of dietary lipids, thereby releasing a range of fatty acids which in turn affect the growth of several bacterial species in vitro. In vivo, we observed differences in hypha formation between the C. albicans wildtype and the lip1-10Δ in a murine model of gut colonization, supporting a role of lipases as an important factor during commensalism. Furthermore, the wildtype strain was able colonize the murine gut more efficiently than the lip1-10Δ mutant, depending on both mice diet (high fat vs standard) and antibiotic treatment. Metagenomic analysis of mice fecal samples showed significant differences between both wildtype- and mutant-colonized mice on the species level, implicating an important role of fungal lipases in shaping the mammalian gut microbiome.
Repurposing glycomimetic drugs as next-generation Anti-Infective to treat fungal infections.

HELMA DAVID, ADLINE PRINCY SOLOMON
SASTRA University, India

Abstract

Biofilm-mediated vaginal candidiasis (VC) is a significant challenge in clinical settings due to the inefficacy of current antifungal therapies to modulate virulence, the development of resistance, and poor penetration into the biofilm matrix. One potential solution is the development of innovative drugs that can modulate fungal virulence/biofilm and potentiate antifungal activity at low concentrations without promoting resistance. The key predisposing factor for candidiasis is uncontrolled sugar levels, which are commonly observed in diabetes as the manifestation of the enzyme α-glucosidase. Interestingly, a structural homolog of α-glucosidase exists in Candida albicans, which is required for its virulence and cell wall composition. We propose using carbohydrate-based drugs (glycomimetics) as anti-infectives to modulate C. albicans pathogenesis and enhance antifungal activity. The crystal structure of α-glucosidase in C. albicans is not experimentally solved. Hence, homology modelling using isomaltose of Saccharomyces cerevisiae (PDB ID: 3AXI) achieved 48.7% sequence similarity between the target and template with 99% query coverage. On virtual screening of FDA-approved glycomimetic drugs on the modelled structure, Acarbose came as the top hit. In vitro studies confirmed that Acarbose inhibited biofilm formation in the range of 90 nM – 200 nM without affecting growth. Our preliminary findings demonstrate that Acarbose can inhibit C. albicans biofilm, virulence, morphogenic switching ability, and host adhesion/invasion and potentiate the effect of existing antifungals at very low concentrations (Filed Patent: 202241054662/23092022), making it a promising candidate for future therapeutic interventions.
THE CAUSES OF AGGREGATION IN CANDIDA AURIS

Dhara Malavia-Jones¹, Matt Edmundson¹, Rhys Farrer¹, Mark Stappers¹, Andy Borman², Elizabeth Johnson², Daan Van Alten³, Neil Gow¹

¹University of Exeter, United Kingdom. ²Public Health England, Bristol, United Kingdom. ³University of Dundee, United Kingdom

Abstract

Candida auris is a multi-drug resistant human fungal pathogen and is recognised as a global threat to human health. Recently, various isolates have been identified which present a unique aggregating phenotype associated with reduced virulence capacity. As a result, dissection of this phenotype has gained significant interest in recent years. In this study, we explored two hypotheses that could account this phenotype. That aggregation is caused by: (i) defects in cell division or separation and/or (ii) that aggregation is mediated by surface adhesin(s). We employed eight clinical isolates from four different clades (I-IV); four of which had aggregating phenotype and four that not form aggregates. Our results demonstrate that aggregating isolates formed a complete septal wall and that separation of daughter cells from their mother cells was normal. We also show that aggregation was influenced by growth temperatures and was reduced or blocked by treatment with Thioflavin-T - an amyloid inhibitor. Furthermore, genome analysis of all eight isolates revealed that certain genes that encode cell wall proteins and GPI-anchor proteins (including Als3 and Als4) were absent in strain-specific manner. It is possible that the absence of these CWPs leads to upregulation of the expression of other amyloid forming CWPs that induce aggregation. Collectively our data suggests that aggregation may be a complex strain-dependent phenomenon involving multiple cellular processes or pathways involving participation of cell surface adhesin(s).
A Candida albicans TLO gene family mutant allows characterisation of alpha (TLOα1), beta (TLOβ2) and gamma (TLOγ11)-clade gene functions

Jessica Fletcher¹, James O’Connor-Moneley¹, Dean Frawley¹, Alessia Buscaino², Samuel Vega Estevez², Matt Anderson³, Derek Sullivan¹, Gary Moran¹

¹Trinity College Dublin, Ireland. ²University of Kent, United Kingdom. ³The Ohio State University, USA

Abstract

Background: Candida albicans is a human commensal and opportunistic pathogen and is the most virulent Candida species. Genomic analysis has highlighted the expansion of the telomere associated ORF (TLO) gene family as unique in C. albicans. In C. albicans there are 15 different members of the TLO family present encoding homologs of Med2. Here we show that clade-representative alpha (TLOα1), beta (TLOβ2) or gamma (TLOγ11) genes are functionally different when expressed in a Δtlo mutant.

Methods: A guide RNA (gRNA) sequence with homology to the TLO genes was used to delete all TLO genes in C. albicans AHY940. The Δtlo strain and derivatives complemented with TLOα1, TLOβ2 or TLOγ11 were subjected to a range of phenotypic tests and RNA-seq analysis in order to characterise phenotypes affected by loss of the TLO gene family.

Results: The Δtlo strain exhibited pseudohyphal morphology, reduced growth rates and hypha production in serum. Clade representative TLOα1, TLOβ2 and TLOγ11 constructs were reintroduced into the null mutant background. HA tagged Tloα1 and Tloβ2 were found to co-immunoprecipitate with Med3 and Med15, to restore a wild-type transcriptome, stress tolerance and growth rates. Uniquely, TLOβ2 expression also showed a dramatic effect on morphology resulting in constitutive true hyphal growth. Tloγ11 did not co-immunoprecipitate with Med3 and Med15 and had limited impact on phenotype and transcriptome.

Conclusion: These data suggest TLO gene clades have distinct functions and that the diversity within the TLO family may modulate transcriptional responses in C. albicans.
Leveraging the antimicrobial potential of bee apitoxin as a promising anti-
Candida agent

Carolina Glória¹,², M Joana Pinheiro¹,², Dalila Mil-Homens¹,², Arsénio M Fialho¹,², Nélson Moura³, Ana
Azevedo¹,², Nuno P Mira¹,²

¹iBB, Institute for Bioengineering and Biosciences, Instituto Superior Técnico – Department of
Bioengineering, Universidade de Lisboa, Lisbon, Portugal., Portugal. ²Associate Laboratory i4HB—
Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco
Pais, 1049-001 Lisboa, Portugal, Portugal. ³ECOAPIS Unipessoal Lda, Portugal

Abstract

Candida Yeasts are leading causative agents of infections in humans, partly due to a continuous
emergence of strains resistant to antifungals used in the clinical setting. This, along with the enhanced
difficulties of obtaining molecules that can restrain fungal growth without causing toxicity for the human
host, has been pressing the search for other alternatives. In this work we investigated the potential of
bee venom (or apitoxin) as an anti-Candida agent. Proteomic characterization of apitoxin shows this is a
very complex mixture of peptides enriched in mellitin and phospholipase A2. Apitoxin exerted a very
potent effect in inhibiting growth of C. albicans and C. glabrata, including of strains showing resistance
to commonly used antifungals (although for these strains growth was less affected). Our results are
consistent with the idea that the anti-Candida potential of apitoxin relies on the melittin present
therein. We could not detect any significant toxic effect of apitoxin when injected in the model wax
Galleria mellonela, including in the concentration range that inhibited growth of Candida. Notably,
treatment of G. mellonela infected with C. albicans with apitoxin fully restrained pathogenesis of the
yeast, without any detectable toxic effects. Among other impacts, the results of this work are expected
to pave the way for the understanding of what are the proteins mediating the anti-Candida effect of
apitoxin, opening new possibilities for the design of bio-based approaches that may leverage the
promising anti-Candida effect observed but that may bypass direct production of apitoxin in the hive.
Herbal Antibiotics as Alternative Promising Anti-Candida drugs

Sujitha Pombala
UCHA Herbs Private Limited, India

Abstract

Every year several million people are suffering with fungal infections ranging from skin to severe cutaneous infections and even potentially life-threatening systemic infections. Among the fungal species, Candida albicans is the most prevalent species in 30-70% of healthy people colonizing the skin and mucosal surfaces of mouth, genitals and intestines. In post antibiotic era, over prescription and over usage of antibiotics, immunosuppressive drugs are causing life threatening bloodstream infections and prolonged hospital stays, higher medical costs, and increased mortality. Hence the quest for non-conventional, safe, effective antibiotic alternatives is of highest priority to address and delay the onset of antibiotic resistance. The plant metabolites are known for medicinal properties especially antifungal activity. In our study we compared and evaluated anti-Candida and MDR activities of several medicinal plants native to India including Nardostachys jatamansi, Boerhavia diffusa, Ocimum sanctum, Mangifera indica, Aegle marmelos, Santalum album, Cyperus rotundus, Tinospora cordifolia, Withania somnifera, Achyranthes aspera, Chrysopogon zizanioides, Hedychium spicatum etc., The extracts were tested for two important parameters: in vitro anti-Candida and anti-biofilm activities. Then the selected potential drugs were evaluated for dynamic microbial growth parameters, in vitro immune responses, activity, stability, solubility and toxicity parameters. Their effect on cell wall integrity, membrane plasticity, biofilm formation is evaluated. The results suggested that N. jatamansi and B. diffusa extracts are likely to be promising drugs for further clinical trials for use against C. albicans and MDR with a positive effect on immune system.
Understanding R-loops and their role in modulating genome plasticity in
*Candida albicans*

Samuel Vega Estevez, Alessia Buscaino

University of Kent, United Kingdom

Abstract

The human fungal pathogen *Candida albicans* is responsible for millions of infections and nearly half a million deaths annually. These disastrous effects on human health have been linked to the ability of *C. albicans* to alter its genome allowing selection of fitter genotypes with advantageous phenotypic traits, such as increased virulence or antifungal resistance.

R-loops play important roles in cell physiology, but can be harmful and cause DNA damage associated to DNA replication stress. R-loops are structures that form during transcription, when the nascent single stranded RNA molecule hybridises with the DNA template, displacing the non-template DNA, to form a DNA:RNA hybrid structure, which is removed by the enzymes RNase H.

To understand how R-loops modulate genome plasticity in *C. albicans* we have used molecular approaches to generate a quadruple RNAse H mutant. This shows increased levels of R-loops by DRIP-qPCR and increased overall genome instability, with important sensitivity to DNA damaging agents.

The effects of these agents on R-loops levels during cell cycle and how increased R-loops might affect genome instability is yet to be determined.
Candida albicans lipids affect the Cdr1p function and localization

RITU PASRIJA, Deepika Kundu
Maharshi Dayanand University, India

Abstract

Background- Candida is a most common pathogenic fungus, causing topical and systemic infections. Its plasma membrane (PM) contains a variety of lipids, like phospholipids, sphingolipids, ergosterol, etc. The common antifungal belongs to the class of azoles, polyenes, and echinocandins and acts on PM lipids and cell walls. However, reports suggest a varying degree of resistance generation against them. One of the primary mechanisms is the over-expression of transporters in the PM, which expels the drugs. These transporters belong to the ABC (ATP Binding cassette) or MFS (Major Facilitator) superfamily of proteins and maintain close interactions with various lipids.

Methods- We deleted the genes of various lipid biosynthesis, including- ERG1 (sterol), PSD1, and PSD2 of phospholipid biosynthesis in a heterologous system, S. cerevisiae and over-expressed the Cdr1p (ABC protein) and Mdr1p (MFS transporter) of C. albicans.

Results– Altering the sterol and phospholipids composition in fungal cells influenced the membrane's fluidity, which increased the drugs' passive diffusion (uptake). Besides that, reduced efflux activity of these transporters was recorded through a fluorescent molecule rhodamin6G. Both these factors increase the cytotoxic concentration of the drugs, resulting in cell sensitivity. Additionally, lipids composition alteration also affected the functioning and targeting of drug transporters.

Conclusion- It can be concluded that besides sterols, phospholipids can also be targeted for designing novel antifungals.


**C. albicans** as a pioneer organism to accelerate polymicrobial biofilm formation on airway management devices

_Campbell Gourlay_1, Ed Deshmukh-Reeves1, Friedrich Mühlschlegel1, Daniel Pentland2

1University of Kent, United Kingdom. 2Kings College London, United Kingdom

**Abstract**

The growth of biofilms on airway management devices (AManD) such as tracheostomy and endotracheal tubing is of significant clinical concern. Biofilm formation on endotracheal tubing alone is estimated to have a global burden of approximately $920m. This is in part due to biofilms of this nature regularly causing ventilator associated pneumonia (VAP). As biofilms found on AManD are polymicrobial in nature, we need to better understand the cell-cell-host-material interactions if we are to develop effective prevention strategies. Here we show how the presence of host factors linked to intubation and the tracheal environment accelerate _C. albicans_ biofilm growth, which in turn helps to recruit bacterial species commonly found within polymicrobial biofilms. Our supporting clinical data suggests that limiting yeast growth greatly reduces biofilm contamination of AManD, leading to an improvement in patient outcome. We hypothesise that _C. albicans_ has evolved to make use of signals found within the trachea to help promote its survival as a component of polymicrobial biofilms that form within the host. In addition, we propose that _C. albicans_ can act as a pioneer organism that promotes polymicrobial biofilm establishment on AManD.
Comparative OMICs analyses of azole-resistant *Candida glabrata* clinical strains reveals adaptive responses involved in the acquisition of the resistance phenotype dependent and independent of CgPdr1

Sara Salazar$^{1,2}$, Maria J Pinheiro$^{1,2}$, Carolina Soares$^{1,2}$, Frédéric Devaux$^3$, Fábio Fernandes$^{1,2}$, Nuno P Mira$^{1,2}$

$^1$iBB, Institute for Bioengineering and Biosciences, Instituto Superior Técnico – Department of Bioengineering, Universidade de Lisboa, Lisbon, Portugal.
$^2$Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal.
$^3$Laboratoire de Biologie Computationnelle et Quantitative, Centre National de la Recherche Scientifique, Institut de Biologie Paris-Seine, UMR 7238, Sorbonne Universités, Université Pierre et Marie Curie Paris, France.

Abstract

The relevance of *C. glabrata* as a human pathogen is linked with its poor susceptibility to azoles as well as its extreme genomic plasticity that drives a rapid acquisition of resistance. A lot of knowledge has been gathered concerning molecular mechanisms underlying tolerance to azoles in *C. glabrata* laboratory strains but much less is known concerning the acquisition of the resistance phenotype in clinical strains. In this work we focused attention on a set of clinical *C. glabrata* strains identified in our lab as being resistant to azoles, including strains recovered from the blood of patients with candidemia but also strains isolated from non-sterile sites, often considered “commensals”. More than 90% of the azole-resistant strains were found to encode gain-of-function variants of CgPdr1, with some of the mutations not being previously described. We will discuss the impact of the found GOF modifications in the biochemical activity of CgPdr1 gathering data from structural simulation, ChIP-SEQ and transcriptomic analyses. The strains encoding wild-type CgPDR1 alleles were found to exhibit, among other features, a particular distribution of sterols in the plasma membrane. This observation was consistent with the detection in these strains of numerous non-synonymous SNPs and higher transcription of genes involved ergosterol metabolism and in intracellular trafficking of sterols. Comparative genomic analyses of all the strains, encoding wild-type or GOF CgPDR1 alleles, identified several candidate proteins likely to contribute for the acquisition of the azole-resistance phenotype suggesting that the landscape of resistance includes, but goes beyond, the CgPdr1-regulon.
The role of Goliath cells in host pathogen-interactions

Iana Kalinina, Duncan Wilson

Medical Research Council Centre for Medical Mycology, University of Exeter, United Kingdom

Abstract

The ability of *Candida albicans* to change morphologies depending on the environment is crucial to its pathogenicity. One of these is the Goliath cell morphology which is induced by limited zinc availability. Zinc is an essential micronutrient and its availability to microbes in the host is restricted by action of nutritional immunity.

We have used a range of tissue culture infection models to dissect the interactions between Goliath cells and host. Goliath cells were more adhesive to human epithelia than yeast cells. However, the greatest level of adhesion observed was between Goliath cells and abiotic surfaces. Extremely robust Goliath cell adhesion was observed under conditions of physiologically relevant flow. This raises the possibility that Goliath cells are suited to colonise indwelling medical devices (such as catheters) from circulation.

Interestingly, Goliath cells lacking the adhesin genes ALS1, ALS1/ALS3, HYR1 or HWP1 exhibited wild type levels of adhesion, indicating that underlying molecular mechanism is fundamentally different to that of hyphae. Rather, we identify a role for cell surface hydrophobicity in mediating Goliath cell adhesion.

Fluorescence microscopy and differential staining demonstrated that Goliath cell hyphal germination and invasion into epithelial was delayed compared to yeasts; however, both cell types went on to form mature mycelia and damage the host tissue at similar levels.

Together our data suggest a model whereby Goliath cells are better equipped to colonise both biotic and abiotic surfaces, particularly under conditions of sheer stress, and that these cells can then proceed to express virulence factors upon induction of hyphae.
Role of pattern recognition receptor signaling in immunity against C. albicans infection in zebrafish

Gursimran Dhillon, Linda Archambault, Drew Brooks, Robert Wheeler

University of Maine, USA

Abstract

Candidemia carries a 40% mortality risk and results in billions in annual healthcare costs. One potential new therapeutic avenue is immunotherapy, but this will require unveiling how our immune system recognizes and responds to Candida albicans. C. albicans is recognized by Toll-like and C-type lectin receptors (TLRs and CLRs), but temporal and spatial patterns of immune activation and fungal clearance are yet to be ascertained at high resolution. I am seeking to define how TLR and CLR signaling regulate the immune response toward Candida infection, leveraging intravital imaging of host and fungal cells in the transparent zebrafish. I am taking advantage of the fact that adapter protein MyD88 is utilized by most TLRs, whereas CLRs utilize CARD9, so inactivation of each will reveal how the whole class of receptors functions. C. albicans infections in mutant zebrafish (missing functional MyD88 and/or CARD9) have been intravitaly imaged and analyzed for fungal pathogenesis and immune activation. Our preliminary data points to an unexpected difference between myd88-/- and wildtype zebrafish immunity: myd88-/- fish survive at higher rates. We also find that NF-kB pathway activation and TNFa expression is not dependent on MyD88. We will be quantifying the overall gene expression of a large panel of immune genes by quantitative PCR. Combining this with longitudinal imaging and survival experiments will hopefully reveal how these pathways affect early immune responses and provide effective immunity (or not) to fungal infection, and this data will hopefully help in the development of immune therapies targeted to fungal infections.
C136

The coordinated activity of Lem3 and Rta3 in Candida albicans regulate the distribution of phosphatidylcholine across the plasma membrane

Pranjali Agarwal¹, Joachim Morschhauser², David Andes³, Sneh Panwar¹

¹School of Life Sciences, Jawaharlal Nehru University, New Delhi, India. ²Institut für Molekulare Infektionsbiologie, Universität Würzburg, D-97080 Wurzburg, Germany. ³Department of Medicine, University of Wisconsin, Madison, USA

Abstract

Background: The asymmetric distribution of phospholipids across the plasma membrane is fundamental to all eukaryotes, and is facilitated by the concerted effort of flippases and floppases.

Methods: Flippase activity was performed using NBD-PC and sodium dithionite. Cells were labeled with 2 µM NBD-PC, and transferred to tubes with and without 25 mM sodium dithionite in SDC and washed with cold SC-azole. Residual MFI of NBD-PC in the presence (F₀) or absence (Fₜₐₜₒₜₜ) of dithionite was measured by flow cytometer. F₀/Fₜₐₜₒₜₜ ratio depicts the percentage of internalized NBD-PC.

Results: A previous study from our laboratory demonstrated that absence of Rta3, a 7-transmembrane receptor protein, resulted in enhanced inward movement (flip) of phosphatidylcholine (PC) across the plasma membrane in C. albicans. Considering that S. cerevisiae Lem3 functions as PC flippase, herein, we sought to explore the relevance of this protein, and its connection with Rta3 in the fungus. We show that the lem3ΔΔ is defective in internalizing a fluorescent structural analog M-C6-NBD-PC, also reflected in a commensurate decrease in PC-specific flippase activity, confirming that Lem3 functions as a flippase. Additionally, perturbed PC asymmetry in the lem3ΔΔ cells reversed azole resistance, and affected in vivo biofilm formation. rta3ΔΔ/lem3ΔΔ exhibited decreased translocation of PC, similar to lem3ΔΔ cells, suggesting that functional Lem3 was contributing to increased translocation of PC in the rta3ΔΔ cells, thus ascertaining the link between Rta3 and Lem3.

Conclusion: Our results show that the combined activity of Rta3 and Lem3 ensure the asymmetric distribution of PC in C. albicans.
RNP granule condensation during *Candida albicans* filamentation

Melissa Tosiano, Fredrick Lanni, Gemma May, C. Joel McManus

Carnegie Mellon University, USA

Abstract

Localization of mRNA to ribonucleoprotein granules (RNPs) such as P-bodies (PBs) and stress granules (SGs) is an integral part of the eukaryotic stress response. RNP condensates have been extensively studied in *S. cerevisiae*, where they are crucial for translational repression and reprogramming the transcriptome during stress. *C. albicans* forms PBs and SGs in response to various stressors, and the PB factor EDC3 has been implicated in heat resistance and filamentation in an auxotrophic *C. albicans* strain (Jung and Kim, 2011). However, many questions remain regarding the roles of RNP condensates in *C. albicans* filamentation and virulence.

To examine RNP formation during *C. albicans* filamentation, we fluorescently tagged canonical SG (PAB1) and PB (EDC3) markers in the SC5314 genome and examined their condensation. In agreement with prior work, we found that PBs and SGs condense in response to acute heat shock. Interestingly, SGs fail to form condensates during physiologically relevant filamentation conditions, while abundant PBs condense along the developing germ tube. Furthermore, copious PBs are present in the hyphae of mature *C. albicans* biofilms. Surprisingly, we found that a homozygous deletion of EDC3 in the prototrophic strain SC5314 did not lead to temperature sensitivity or a filamentation defect under strongly inducing conditions. We are currently confirming that edc3ΔΔ causes a loss of PB formation in SC5314. We are also examining the impact of deleting EDC3 in diverse strains and interrogating filamentation defects under a variety of conditions. These studies will clarify the function of PBs and SGs in *C. albicans* filamentation.
Phenotypic Screening of the Pandemic Response Box from Medicines for Malaria Ventures to Identify Drugs with Novel Antifungal Activity against Candida albicans and Candida auris

Olabayo Ajetunmobi\(^1\), Nathan Wiederhold\(^2\), Laura Najvar\(^3\), Thomas Patterson\(^3\), Jose Lopez-Ribot\(^1\)

\(^1\)Department of Molecular Microbiology & Immunology, and South Texas Center for Emerging Infectious Diseases, The University of Texas at San Antonio, San Antonio, Texas, USA. \(^2\)Fungus Testing Laboratory, The University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA. \(^3\)Division of Infectious Diseases, Department of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA

Abstract

Background: Candida spp. are opportunistic yeasts capable of forming biofilms, which contribute to resistance, increasing the urgency for new effective antifungal therapies. Repurposing existing drugs could significantly accelerate the development of novel therapies against candidiasis.

Methods: We screened the Pandemic Response Box containing 400 diverse drug-like molecules active against bacteria, viruses, or fungi, for inhibitors of C. albicans and C. auris, both under biofilm-growing conditions. Initial hits were identified based on the demonstration of >70% inhibitory activity. Dose-response assays were used to confirm the antifungal activity of initial hits and establish their potency. The spectrum of antifungal activity of the leading compounds was determined against a panel of medically important fungi, and the in vivo activity of the leading repositionable agent was evaluated in murine models of C. albicans and C. auris systemic candidiasis.

Results: The primary screening identified 20 hit compounds, and their antifungal activity and potency against C. albicans and C. auris were validated using dose-response measurements. From these experiments, the rapalog everolimus, emerged as the leading repositionable candidate. Everolimus displayed potent antifungal activity against different Candida spp., but more moderate levels of activity against filamentous fungi. Treatment with everolimus increased the survival of mice infected with C. albicans, but not those with C. auris.

Conclusion: The screening of the pandemic response box resulted in the identification of several drugs with novel antifungal activity, with everolimus emerging as the main repositionable candidate. Further in vitro and in vivo studies are needed to confirm its potential therapeutic use.
Validation of Scoring Systems and (1, 3)-β-D-Glucan Assay for Early Diagnosis of Invasive Candidiasis in High-Risk Non-Neutropenic Patients in Siriraj Hospital: A Prospective Cohort Study

Supicha Ongkittikul¹, Methee Chayakulkeeree¹, Ariya Chindamporn²

¹Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. ²Faculty of Medicine, Chulalongkorn University, Thailand

Abstract

Background: Early diagnosis and empirical therapy for invasive candidiasis is important for improved survival.

Objective: To evaluate the Siriraj Canidiasis Assessment Score (SiCAS), Leon’s Candida score (LCS) and serum (1,3)-β-D-glucan (BDG) in predicting invasive candidiasis in non-neutropenic patients.

Methods: The prospective cohort study was performed in Siriraj Hospital between January 2014 and February 2015. Non-neutropenic patients who were suspected invasive candidiasis were enrolled. Both SiCAS and LCS were calculated and serum BDG was performed.

Results: Of the 196 patients enrolled, 88 (44.9%) were invasive candidiasis, 53 (27%) with candidemia and 35 (17.9%) with deep organ candidiasis. Risk factors of invasive candidiasis were receipt parenteral nutrition (adjusted OR, 3.5) and concurrent bacteremia (adjusted OR, 5.4). The sensitivity of SiCAS for predicting of invasive candidiasis with a cut-off ≥0 was 91%, specificity 13%, positive predictive value (PPV) 46% and negative predictive value (NPV) 64%. For LCS with a cut-off ≥3, sensitivity was 71.6%, specificity 31.5%, PPV 46% and NPV 57.6%. Using a cut-off ≥ 80 pg/mL, the percentage of invasive candidiasis patients with positive serum BDG (76.2%) was significantly higher than those with negative result (23.8%; p<0.001). The serum BDG with cut-off≥80 pg/ml yielded sensitivity 76%, specificity 51%, PPV 55%, and NPV 73%.

Conclusions: Serum BDG provided a moderate to good accuracy for diagnosis of invasive candidiasis, while both SiCAS and LCS showed a fair to moderate accuracy to predict invasive candidiasis in high-risk non-neutropenic patients in our setting and should be used together with serum BDG.
Putative GPI-anchored protein Fgr41 impacts β(1,3)-glucan exposure and cell wall organization in *C. albicans*.

Ainsley King, Andrew Wagner, Stephen Lumsdaine, Elise Phillips, Todd Reynolds

University of Tennessee, Knoxville, TN, USA

Abstract

*Candida albicans* is a pathogenic fungus that can cause life-threatening infections. One potential method for treating these infections is to increase immune recognition and clearance of the pathogen. The cell wall of *C. albicans* contains β(1,3)-glucan, which triggers a robust pro-inflammatory response, making it a good target to leverage for immunotherapy. However, an outer layer of mannosylated proteins covers, or masks, most of the β-glucans, hindering immune recognition. Our lab previously reported that the putative adhesin Fgr41 plays a role in cell wall organization and masking of β(1,3)-glucan. *FGR41* is downregulated during treatments that cause unmasking, such as treatment with the echinocandin caspofungin and hyperactivation of the MAP3K *STE11*. We therefore disrupted *FGR41* and discovered that *fgr41Δ/Δ* exhibits increased unmasking, especially in the bud necks and bud scars, and has attenuated virulence in mice that is dependent on the presence of a functional host immune system. Its unmasking trends were mirrored in TNF-α release from macrophages. Conversely, overexpression of *FGR41* causes a reduction in unmasking induced by *STE11* hyperactivation. The *fgr41Δ/Δ* mutant also has reduced separation of budding cells, a phenotype that is shared with another reported unmasking mutant *eng1Δ/Δ*. Eng1 codes for a cell wall β-glucanase; when we overexpressed *ENG1* in the hyperactive *STE11* mutant, it caused a reduction in unmasking. Moreover, when *ENG1* was overexpressed in the *fgr41Δ/Δ* mutant, unmasking was also reduced. We are currently exploring the mechanism by which Fgr41 regulates unmasking and interactions it may have with other cell wall proteins.
Interkingdom Interactions Between *Pseudomonas aeruginosa* and *Candida auris*

Charlton Lam, Katarzyna Danis-Wlodarczyk, Daniel Wozniak
Ohio State University, USA

**Abstract**

*Candida auris* has been isolated from wounds and bronchoalveolar lavage, common sites also for *Pseudomonas aeruginosa* infections. While antagonism between *Candida albicans* and *P. aeruginosa* has been demonstrated previously, interactions between *C. auris* and *P. aeruginosa* are vastly understudied. Using colony counting, microscopy, and metabolic studies, we examined differences in the population dynamics of *C. auris* grown planktonically or in biofilms in the presence and absence of *P. aeruginosa*. Growth of a *C. auris* strain from the recently discovered Clade V was inhibited tenfold when grown planktonically in the presence of *P. aeruginosa* PAO1K in comparison to its growth as a monoculture. Subjecting Clade V to PAO1K conditioned media nearly completely halted growth planktonically, suggesting that a soluble factor released by *P. aeruginosa* is responsible for this effect. The conditioned media had a decreased inhibitory effect on strains from other *C. auris* clades, which otherwise demonstrated minimal antagonism in coculture studies with *P. aeruginosa*. Similarly, the conditioned media had a limited inhibitory effect on *C. albicans* SC5314. Both heat-treated and proteinase K-treated conditioned media yielded similar growth patterns across all strains tested. These data suggest that *P. aeruginosa* produces and releases a heat-stable and proteinase K-resistant soluble factor that inhibits the growth of *C. auris* Clade V and that the growth inhibition is both species- and strain-dependent.
A Cautionary Tale: Genetic background alters the role of SIR2 in *Candida albicans* phenotypic switching

Andrew Woodruff, Matthew Anderson

The Ohio State University, USA

Abstract

Transition among cell states is required for pathogenesis and alters fitness across host niches in *Candida albicans*. Epigenetic regulatory mechanisms often underlie cell state transitions, including interconversion between the ‘sterile’ white and mating-competent opaque states. Prior work suggested a role for the sirtuin histone deacetylase SIR2 in *C. albicans* phenotypic switching but was incompletely defined. To clarify its role in white-opaque switching, we assayed a previously constructed BWP17 sir2Δ/Δ strain set alongside a newly constructed SC5314 sir2Δ/Δ strain set. After incubation for seven days on solid SCD medium at room temperature, the BWP17 sir2Δ/Δ mutants but not the SC5314 sir2Δ/Δ mutants displayed increased opaque colony formation. This is unlikely to reflect an altered SIR2 functionality as both lineages replicated the previously demonstrated role for this gene in subtelomeric silencing. To better understand this discrepancy, strains containing the auxotrophic mutations and IRO1 disruption that define BWP17 were built in SC5314 but did not lead to increased white-opaque switching either alone or in combination with sir2Δ/Δ. Furthermore, reconstruction of sir2Δ/Δ in the ‘wildtype’ BWP17 background did not recapitulate the elevated switching phenotype. Interestingly, additional aspects of mating, such as development of mating projections and mating efficiency on solid Spider medium, were altered in the original BWP17 sir2Δ/Δ mutants that could be partially complemented by wildtype SIR2. These data definitively demonstrate that SIR2 does not regulate white-to-opaque switching in SC5314 under the assayed conditions. Previously observed associations likely reflect epistatic interactions between SIR2 and background mutations or changes in the specific BWP17-constructed lineage.
Conducting genetic interaction analysis in *Candida albicans* by using CRISPR-Cas9-based gene drive to target stress response genes

Viola Halder, Prakritie Shahi, Rebecca Shapiro

University of Guelph, Canada

Abstract

*Candida albicans* is a commensal organism, typically found in the oral mucosa, the gut, the vaginal mucosa, and the skin of humans and other mammals. *C. albicans* is also an opportunistic fungal pathogen that may cause superficial to severe invasive and systemic infections, depending on the status of an individual’s immune system.

Cells need to adapt to environmental stressors to survive, and this ability is dependent on the speed and robustness of their stress responses. For *C. albicans*, stress response factors can also modulate antifungal drug resistance, as antifungal drugs can impart different forms of stress on the fungal cell. Understanding *C. albicans*’ stress responses would be prudent in novel antifungal drug discovery.

We exploit a genetic approach using the CRISPR-Cas9-based genome editing platform to map out genetic interactions between fungal genes, targeting stress response genes with roles in fungal cell survival. The proposed research aims to dissect the genetic interactions underpinning *C. albicans*’ response to diverse stressors. This proposed research will use cutting-edge genomic technologies to explore the genetic mechanisms by which *C. albicans* survive amidst a constantly changing environment. Leveraging this platform, we will have the ability to gain previously inaccessible biological insight into the complex genetic interaction networks in *C. albicans*, and discover fundamental processes in fungal biology, including stress tolerance.
B145

CHROMATIN REMODELERS AND THEIR CONTRIBUTION TO THE FITNESS AND DRUG RESISTANCE of Candida auris

Maria Mantilla\textsuperscript{1,2}, Adnane Sellam\textsuperscript{1,2}

\textsuperscript{1}Montreal Heart institute, Canada. \textsuperscript{2}Department of Microbiology-Infectious Disease and Immunology, Université de Montréal, Canada

Abstract

Candida auris represents a serious global health threat that can cause systemic infections in patients with pre-existing health conditions, with a negative prognosis due to resistance to the commonly used antifungals. It is thus of utmost urgency to understand the pathogenic behavior and discover new drug targets for novel antifungal therapeutics against this superbug. One possibility to achieve this is to target transcriptional regulatory networks, such as chromatin remodelers (CRs). The CR modify chromatin structure which consequently allows the transcription signals to reach their destination in the DNA. The study of CRs networks of fungal pathogens is an attractive research topic that can help to fight them by targeting transcriptional circuits instead of individual effectors of virulence or drug resistance. We systematically deleted genes of the CRs of C. auris (Snf2, Rsc4, Ino80, Issw2, Issw1a, Issw1b, and Chd1) and undertook comprehensive phenotypic profiling in different conditions reflecting the host environment, including different carbon sources, temperature, osmostress, and pH. CRs inactivation was performed in a different genetic backgrounds including both sensitive and resistant strains. Our data underscore the importance of CRs control for metabolic flexibility (snf2, chd1, and isw2) and resistance to different stresses including antifungal challenges. Overall, our results suggest that CRs play a role in the resistance to antifungals, as well as in the adaptation to the different types of conditions evaluated.
Acetylation of Eaf3, a chromodomain protein, by the Gcn5 lysine acetyltransferase is required for virulence of Candida albicans

Raju Shivarathri¹, Sabrina Jenull², Manju Chauhan¹, Phillip Penninger², Andriy Petryshyn², Michael Tscherner², Markus Hartl², Karl Kuchler², Neeraj Chauhan¹

¹Center for Discovery and Innovation, USA. ²Medical University of Vienna, Austria

Abstract

Fungal virulence is regulated by a tight interplay of transcriptional control and chromatin remodeling, the latter including reversible histone acetylation that plays a critical role in the regulation of gene expression. However, the role of reversible lysine acetylation in fungal pathogenesis remains poorly understood. We present data that suggest lysine acetylation of proteins is a major mechanism that regulates morphogenesis in C. albicans. We performed proteomics experiments to identify acetylated proteins in C. albicans. Using this approach, we discovered that proteins involved in diverse biological processes, including pathogenesis, were acetylated in C. albicans. A total of 1442 acetylation sites on 705 proteins were identified in wild-type cells. Our results indicate that acetylation preferentially targets metabolic enzymes in glycolysis, TCA cycle and oxidative phosphorylation. Furthermore, we combined quantitative proteomics to determine the Gcn5 mediated histone and non-histone protein acetylation in C. albicans. Gcn5 is a paradigm fungal lysine acetyltransferase (KAT) essential for fungal virulence and antifungal drug resistance. Our data reveal acetylation of H2BK and H3K is dependent on Gcn5. Interestingly, several proteins in MAPK pathways were found to be acetylated in a Gcn5 dependent manner. From our acetylome data we have identified Eaf3, a subunit of NuA4 histone acetyl transferase complex, as a Gcn5 acetylation target. Importantly, Eaf3 is required for echinocandin resistance, filamentation and virulence of C. albicans. Collectively, our studies provide a framework for understanding of how KATs/KDACs impact fungal virulence by regulating both histones and non-histone targets during infection and may aid in antifungal drug discovery.
Characterizing *C. albicans* virulence mechanisms in the catheterized bladder environment.

Alyssa La Bella, Ana Flores-Mireles, Felipe Santiago-Tirado

University of Notre Dame, USA

Abstract

Background: Catheter-associated urinary tract infections (CAUTIs) are the most common hospital-acquired infection and result in increased morbidity, mortality, and an annual cost of ~$400 million in the US alone. Despite *Candida* spp. – specifically *Candida albicans* – being the second most common CAUTI uropathogen (17.8%), the details of these infections are poorly understood.

Methods: Several *in vitro* assays such as biofilm formation, morphology assessment, and gene expression quantification were performed under urine conditions in the presence/absence of fibrinogen (Fg) for 48 hours using a variety of WT and mutant strains made in laboratory and clinical isolates. These were complemented with *in vivo* assays, where mice were catheterized and infected with WT or mutant strains, including strains overexpressing the adhesin ALS1. After 24 hours, the mice were euthanized and organs harvested for CFU enumeration, histological analysis, and determination of fungal gene expression.

Results: We found that the catheterized bladder environment triggers *Candida* virulence programs and robust biofilm formation through Efg1-dependent hyphal morphogenesis and Als1, an Efg1-downstream effector. Additionally, we show that the adhesin Als1 is necessary for *in vitro* and *in vivo* biofilm formation dependent on the presence of Fg, which is abundantly released in the bladder during catheterization. Furthermore, in the presence of Fg, overexpression of ALS1 led to enhanced colonization and dissemination, while deletion of ALS1 reduced both outcomes during CAUTIs.

Conclusion: Our study characterizes fungal virulence mechanisms and fungal biofilm formation during CAUTI for the first time and ultimately unveils the mechanism that contributes to fungal CAUTI.
C149

Investigation of a sterol signaling pathway required for azole-induced Set4 expression in *Candida glabrata*

Justin Gregor, Scott Briggs

Purdue University, USA

Abstract

*Candida glabrata* infections are a growing concern in clinical settings due to the pathogen’s intrinsic resistance to azole drugs and ability to rapidly develop clinical drug resistance. Azole drugs target lanosterol 14-α demethylase (Erg11), a key enzyme in the ergosterol biosynthesis pathway, and inhibiting Erg11 leads to depletion of ergosterol and a buildup of intermediate and toxic sterols. Our lab has determined that in both *Saccharomyces cerevisiae* and *C. glabrata*, Erg3 is an enzyme in the ergosterol biosynthesis pathway whose loss is associated with Upc2-mediated upregulation of *SET4*. This is notable since overexpression of Set4 leads to azole hyper-susceptibility. In the presence of azoles or when Erg3 is absent, ergosterol is depleted and the cell resorts to side pathways involving Erg4, Erg5, or Erg6 to produce intermediate sterols. Because the expression of Set4 is increased when Erg3 is absent, we hypothesized that these precursor sterols accumulate when ergosterol is depleted. These intermediate sterols then act as a sterol signaling pathway to induce Upc2-mediated Set4 expression. To determine if intermediate sterols induce *SET4* expression, we probed the ergosterol biosynthesis pathway by generating a series of CRISPR-mediated single and double deletion of genes required for ergosterol biosynthesis pathway. Because Set4 overexpression results in azole hypersensitivity, the identification of intermediate sterols that induce the expression of Set4 would lead to azole susceptibility, potentially providing an alternative therapeutic for combating azole drug resistance.
High-level fluconazole resistance in *C. parapsilosis* clinical isolates driven by combined effects of Y132F substitution in Erg11 and an activating G650E substitution in Tac1.

Laura A. Doorley1,2, Jeffrey M. Rybak1, Katherine S. Barker1, Qing Zhang1, P. David Rogers1

1St. Jude Children's Research Hospital, USA. 2University of Tennessee Health Science Center, USA

Abstract

Background:

*Candida parapsilosis* is the most common non-*albicans* agent causing candidemia in neonatal and pediatric populations. The Y132F substitution in CpErg11 is frequently identified in clinical isolates with varied fluconazole (FLU) MICs. We previously identified CpCDR1 overexpressing isolates, Cp35 and Cp38, contained mutations leading to CpTac1<sup>G650E</sup> and CpErg11<sup>Y132F</sup> substitutions.

Methods:

Sanger sequencing was used to identify *CpTAC1*, *CpERG11*, and *CpCDR1C* sequences in a collection of *C. parapsilosis* clinical isolates. A plasmid-based Cas9 system was used to make genetic modifications in *CpTAC1* and *CpERG11*. FLU MICs were determined by broth microdilution and read visually for 50% growth reduction at 24 hours. RNA sequencing and transcriptional analysis were performed using Illumina NovaSeq and CLCgenomics workbench.

Results:

Introduction of CpErg11<sup>Y132F</sup> into susceptible isolates Cp13, Cp23, and Cp3 resulted in a four- to eight-fold increase in FLU MIC, however, measurements did not surpass clinical breakpoints for resistance. Correction of CpTac1<sup>G650E</sup> to CpTac1<sup>WT</sup> in Cp35 and Cp38 resulted in 32-fold decreases in FLU resistance. Transcriptional analysis of Cp35 and Cp38 compared to their derived CpTac1<sup>WT</sup> strains revealed elevated expression of three ABC transporter genes, *CpCDR1*, *CpCDR1B*, and CPAR2_300010, a previously classified pseudogene. Sanger sequencing for CPAR2_300010 revealed a K1144fs, resulting in a full ABC transporter sequence. Constitutive overexpression of *CpCDR1*, *CpCDR1B*, and *CpCDR1C* (CPAR2_300010) in Cp13 increased FLU MICs 16-, 16-, and 4- fold, respectively. Combined disruption of these three transporters in Cp35, decreased FLU resistance by 4-fold.

Conclusion:

High-level fluconazole resistance in *C. parapsilosis* can be driven by CpErg11<sup>Y132F</sup> working in concert with an activating mutation in *CpTAC1*. 


Is the Non-Homologous End Joining (NHEJ) repair pathway implicated in drug resistance in the opportunistic pathogen *Candida glabrata*? A laboratory microevolution experiment

Laetitia Maroc¹, Sydney Johnson², Rebecca Shapiro¹

¹University of Guelph, Canada. ²University of Concordia, Canada

Abstract

*Candida glabrata* acts as an opportunistic pathogen in immunocompromised patients and accounts for approximately 15–25% of invasive Candida infections. This yeast is associated with a high frequency of drug resistance and, particularly, shows a great ability to adapt toazole and echinocandin antifungal agents which are widely used for Candida treatment. Known mutations involved in azole and echinocandin resistance imply, respectively, mutations in *PDR1*, which encodes a transcriptional regulator of drug efflux pumps, and in the *FKS* genes, implicated in the biosynthesis of a major structural component of fungal cell walls. However, mechanisms leading to drug adaptation are still poorly understood. Non-Homologous End Joining (NHEJ) is a double-strand break repair pathway which can introduce mutations in the genome and, to our knowledge, its implication in drug resistance has never been studied. In this work, we used a laboratory micro-evolution experiment to better assess adaptation of *C. glabrata* to fluconazole (azole) and caspofungin (echinocandin) as well as to decipher the implication of NHEJ in drug resistance. WT and *lig4Δ* (NHEJ mutant) *C. glabrata* strains were exposed for prolonged periods to twice their MIC50 (Minimum Inhibitory Concentration required to inhibit the growth of 50% of cells) of fluconazole and caspofungin. Our preliminary results show that *C. glabrata* can adapt quicker to fluconazole than to caspofungin in both the WT and mutant strains. The mechanisms contributing to this adaptation remain to be unveiled and will tell us if the WT and *lig4Δ* strains’ adaptation followed the same or different evolutionary pathways.
C152

Neutrophil Swarming is Crucial for Defense to Oral Candidiasis

Isolde Rojas, Rohitashw Kumar, Mira Edgerton

University at Buffalo, USA

Abstract

Objective: Neutrophils are key players in protection against C. albicans (Ca) during oral candidiasis (OPC). Cortisone immunosuppression (IS) increases Ca oral infection and mortality in mice, despite abundant neutrophil accumulation at later stages of infection. Neutrophils organize into three-dimensional clusters, known as swarms, for microbial killing that we hypothesize is a mechanism to control Ca infection.

Methods: Mice (C57BL/6J) were infected sublingually with Ca and IS induced by cortisone injection. IS and immunocompetent (IC) mice were sacrificed day-post-infection dpi1, dpi3, and dpi5, and tongue epithelium (Ep) collected for CFU quantification, swarm analyses, immunohistochemistry and Multiplex ELISA assays.

Results: IS and IC tongues had equal Ca CFU at dpi1. IC Ep had large swarms of inflammatory cells that were Ly6G+, myeloperoxidase+ (MPO+) and in some cases Arg1+ subjacent to invading Ca that was cleared after dpi1. CD163+ macrophages were not found in Ep. IS tongues dpi1 had an absence of neutrophil infiltration, despite Ca invasion. IS tissue dpi3 and dpi5 had significantly increased CFU surrounded by a disorganized neutrophilic infiltrate. BLT1, a marker of swarming, was only detected in IC mice. Large swarming areas (>8,000mm²) were observed in IC mice on dpi1, whereas IS mice showed reduced swarming areas (<2,000mm²) from dpi3-5 (p<0.01). IL-1β, G-CSF, CXCL1 and CXCL2 were higher in IC than IS mice on dpi 1 (p<0.01), whereas IS had increased expression dpi3 and dpi5 (p<0.05).

Conclusions: Neutrophil swarming is a critical defense mechanism against Ca during OPC that is disrupted by Glucocorticoid-induced IS leading to impaired Ca clearance.
Antivirulence drug discovery with a new luciferase-based host damage reporter

Raghav Vij¹, Millen Tesfamariam¹, Sophie Tröger¹, Amninder Kaur², F.P. Jake Haeckl², Verena Trümper¹, Rolf Müller², Axel A. Brakhage¹, Olaf Kniemeyer¹, Bernhard Hube¹, Sascha Brunke¹

¹Leibniz Institute for Natural Product Research and Infection Biology-Hans Knoell Institute, Germany.
²Helmholtz Institute for Pharmaceutical Research Saarland, Germany

Abstract

The antifungal armamentarium is limited, and the development of resistances against fungicidal substances is close to an evolutionary inevitability. To address this problem, we have turned to screening for antivirulence compounds, which act against Candida albicans’ ability to damage host cells, but do not affect growth or survival – thus alleviating the strong selection pressure of fungicidal and fungistatic drugs.

For our screen, we have developed a new pipeline. At the heart is the testing of compounds directly on C. albicans infecting host cells. This allows to screen out ineffective substances as well as those which are toxic to the host. Secondly, we created a new, efficient read-out for host cell damage.

For the substances, we tapped the mostly unexplored wealth of metabolites produced by soil-dwelling myxobacteria, which are often in competition with environmental fungi. In our ongoing screen, we have analyzed ≈2,800 extracts and compounds from myxobacteria, and ≈1,500 compounds from other sources for antivirulence and antifungal activities in the in vitro infection context.

On the host side, we developed and thoroughly validated a new cell damage readout. We constitutively express a modified luciferase in several commonly used host cell lines. By detecting the luciferase activity, we have created an efficient, inexpensive, and reliable system to detect host damage in vitro.

With this combined screening pipeline, we identified and confirmed several compounds that protected mammalian epithelial cells from C. albicans damage. A multi-omics characterization of the modes of action of these potential antivirulent and antifungal compounds is currently under way.
Transcriptional regulation of the synthesis and secretion of farnesol in *Candida albicans*

Daniel Gutzmann, Jaxon Kramer, Brigid Toomey, Cory Boone, Audrey Atkin, Kenneth Nickerson

University of Nebraska-Lincoln, USA

Abstract

*Candida albicans* is an opportunistic pathogen and an efficient colonizer of human gastrointestinal tracts and skin. *C. albicans* exhibits morphological plasticity depending on environmental cues. This ability to switch between yeast and filamentous morphologies is associated with virulence and pathogenicity. One regulator of this switch is the quorum sensing molecule farnesol. Farnesol is produced by *C. albicans* cells continuously throughout growth, but how its synthesis and secretion are regulated is unclear. To address this gap, we used our improved assay for farnesol quantification of whole cultures, pellets, and supernatants, to screen the Homann transcription factor knockout library of 165 mutants for differences in farnesol accumulation and localization. We found that all mutants produced farnesol with an average of 9.2X more farnesol localized to the pellet in most mutants. 19 mutants had significant differences in accumulation. Ten mutants produced more farnesol than their SN152 parent while nine produced less. Seven mutants exhibited greater secretion of farnesol while four exhibited less. Several of these mutants have known roles in white/opaque switching and cell cycle progression such as the farnesol overproducing mutants *ahr1* and *swi4*. To discover what genes may be responsible for this difference in farnesol accumulation, we RNA sequenced three mutants with lower farnesol production and identified *IFD6* and *CWH8* as gene candidates. *CWH8* is of particular interest given the structural similarity of farnesyl and dolichyl pyrophosphate. The transcription factors identified in this screen further understanding of the gene regulatory networks controlling morphogenesis, as well as farnesol regulation.
Grf10 regulates the response to copper, iron, and phosphate in *Candida albicans*

Ronda Rolfes, Sylvia Arnold, Tanaporn Wangsanut
Georgetown University, USA

Abstract

The pathogenic yeast, *Candida albicans*, and other microbes must be able to handle drastic changes in nutrient availability within the human host. Copper, iron, and phosphate are essential micronutrients for microbes that are sequestered by the human host as nutritional immunity; yet high copper levels are employed by macrophages to induce toxic oxidative stress. Grf10 is a transcription factor important for regulating genes involved in morphogenesis (filamentation, chlamydospore formation) and metabolism (adenylate biosynthesis, one-carbon metabolism). The *grf10Δ* mutant exhibited resistance to excess copper in a gene dosage dependent manner but grew the same as the wild-type in response to other metals (calcium, cobalt, iron, manganese, and zinc). Point mutations in the conserved residues D302 and E305, within a protein-interaction region, conferred resistance to high copper and induced hyphal formation similar to strains with the null allele. The *grf10Δ* mutant misregulated genes involved with copper, iron, and phosphate uptake in YPD medium and mounted a normal transcriptional response to high copper. The mutant accumulated lower levels of magnesium and phosphorus, suggesting that copper resistance is linked to phosphate metabolism. Our results highlight new roles for Grf10 in copper and phosphate homeostasis in *C. albicans* and underscore the fundamental role of Grf10 in connecting these with cell survival.
A comprehensive strategy for identifying Candida albicans genes and biologic processes that contribute to pathogenesis of intra-abdominal candidiasis (IAC).

shaoji cheng1, Kevin Michael Squires2, ming-Hong Nguyen2, Cornelius Joseph Clancy2,3

1University of Pittsburgh, USA. 2University of Pittsburgh, USA. 3VA Pittsburgh, USA

Abstract

Background. Pathogenesis of IAC is poorly understood.

Methods. We used RNA-Seq to measure C. albicans SC5314 gene expression during early peritonitis (30-min), late peritonitis (24-hr) and abscesses (48-hr) of mice. ≥2-fold differences were significant (FDR ≤0.01). We screened duplicate signature-tagged, homozygous deletion libraries for 165 C. albicans transcription factors (TFs) in 72-hr abscesses.

Results. The 50 genes most highly expressed during early peritonitis were associated with pH (e.g., RIM101), oxidative stress responses (e.g., SOD4-6), and adhesion/hyphal growth (e.g., ALS3, HWP1, ECM331, SAP6). The corresponding 50 late peritonitis genes were associated with neutrophil/macrophage responses and nutrient acquisition (glyoxylate cycle, fatty acid β-oxidation, iron homeostasis). Responses within abscesses included DNA damage and iron metabolism. 21 TF mutants were significantly attenuated in abscesses. Over-represented biologic processes were pH response, biofilm, filamentation, echinocandin and copper responses. C. albicans transitions from pH 8 (peritonitis) to 6.5 (abscesses) during IAC. Virulence-mediating pH TFs were RIM101, STP2 (alkaline), ASH1, SFL1, SFL2 (neutral), MNL1, SKO1, PHO4 (weak acid), and CSR1 (acid). Null mutants for genes involved in adhesion (ALS1, ALS3), transport (OPT8, SGE11), biofilm (ZCF23), DNA damage (RFX1, RFX2, DDI1), cell wall responses (DAP2), copper metabolism (CCC2), alkaline pH (RIM101, SAP5) and weak acid (MNL1, SKO1, PHO4) were attenuated for virulence during peritonitis and/or abscesses.

Conclusions. C. albicans responds to different environmental conditions during IAC. Numerous environmental response genes make temporal-spatial contributions to virulence. pH response regulators RIM101 and MNL1 contribute to virulence during peritonitis and abscesses, in part by regulating SAP5 and PHO4/SKO1, respectively.
Candida auris in positive clinical cultures demonstrate genetic and phenotypic diversity.

shaoji cheng¹, Hassan Badrane², Anthony Michael Newbrough², Cornelius Joseph Clancy², minh-hong nguyen²

¹university of pittsburgh, USA. ²university of pittsburgh, USA

Abstract

Background: We recently showed within-host genetic and phenotypic diversity of Klebsiella pneumoniae, Candida albicans and C. glabrata from blood cultures (BCs) of patients with bloodstream infections (BSIs). We assessed within-host diversity of Candida auris (Cau) from BC of a patient with central-line-associated-BSI

Methods: We performed Illumina sequencing on ≥10 strains from positive BCs and characterized phenotypes.

Results: Myriad colony sizes were evident on CHROMagar on day 4. We isolated 22 large and 19 small colonies. Strains (clade III) differed by median 356 SNPs, roughly comparable to differences among longitudinal strains from Cau-colonized individuals in some studies. Large and small colony strains intermixed in the phylogenetic tree. 90 genes carried mutations in ≥1 and <41 strains. Both large and small colony strains had mutations in adhesion, MFS transport, oxidative stress, mismatch repair genes. Only large colonies had mutations in cell division, vesicular transport, cytoskeleton genes. Only small colonies had mutations in electron transport, vacuolar sorting, tubulin genes. Small colony strains were not respiration-deficient by various assays. Median liquid doubling-time, adhesion and FACS cell size of large and small colony cells were similar, but intra-group ranges were wide (79-129 mins, 76-171 mins; 25%-130%, 21%-120%; 20,544-47,084). All strains were azole-resistant; 2 (1 large/1 small) were echinocandin-resistant. In time-kills, micafungin killed a representative echinocandin-susceptible large strain, but not an echinocandin-susceptible small strain.

Conclusions: Cau from BC of a patient with BSI were genetically and phenotypically diverse, including in echinocandin-resistance not recognized by the clinical lab. Strain diversity from a second patient is being defined.
B158

GO term-focused expansion of the GRACE (Gene Replacement and Conditional Expression) library

Linda Archambault, Ci Fu, Clara Hick, Shiarati Sara, Emma Lash, Isabel Sternthal, Mahrukh Fatima, Aiman Farheen, Miaoxi Liu, Nicole Robbins, Leah Cowen

University of Toronto, Canada

Abstract

The normally harmless commensal yeast, *Candida albicans*, presents a serious health threat to the immunocompromised. The low number of effective antifungals, coupled with emergence of drug-resistant strains, make the search for new treatment options urgent. To address this need through a functional genomics strategy, we are continuing to develop the *C. albicans* Gene Replacement and Conditional Expression (GRACE) library, in which one allele of a gene is replaced by an auxotrophic marker while the second allele is under the control of a tetracycline-repressible promoter. GRACE strains have allowed the screening of large numbers of *C. albicans* genes for essentiality, drug sensitivity, and virulence traits such as morphogenesis and interactions with host immune cells. Over the past few years, we have brought the collection from ~40% to over 60% coverage of the *C. albicans* genome. Recently, we turned our focus to generating sets of genes important for basic cellular functions and virulence traits as defined by Gene Ontology (GO) terms. Conditional mutant collections corresponding to 10 GO terms are now 80-100% complete, and screening of these resources unveiled novel functions for previously uncharacterized genes. For example, *SWI1*, a member of the adhesion GO term group, functions as a broad-spectrum drug-sensitivity regulator, and *CET1*, a member of the phosphatase GO term group, plays a previously undescribed role in filamentation. These findings demonstrate how functional analysis of *C. albicans* genes can expand the set of fungal targets for therapeutic intervention as well as uncover fascinating biological insights into an important human fungal pathogen.
A 1,000-year-old remedy has potent fungicidal activity against a range of *Candida* species

Jessica Furner-Pardoe\(^1\), Freya Harrison\(^2\), Mark Sutton\(^1\), Charlie Hind\(^1\)

\(^1\)UKHSA, United Kingdom. \(^2\)University of Warwick, United Kingdom

Abstract

*Candida* spp. can be responsible for a number of important diseases including candidiasis. Our work has focused on investigating if a historical remedy, shown to be potent against bacterial infections, is also active against *Candida*. This 1,000-year-old remedy comes from an early medieval manuscript, and consists of garlic, onion, wine and bile - all of which has been stored for 9 days. Previous work has shown the remedy is effective at killing a broad range of bacteria and has minimal toxicity *in vitro*, *in vivo* and in Phase I safety trials. Here we show that this remedy has potent fungicidal activity against 21 *Candida* strains (including *C. auris*, *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis* and *C. parapsilosis*), all tested according to EUCAST guidelines. Less than 1% of the remedy was required to inhibit the strains growth, and <2% was required to kill all *Candida* strains. Interestingly, this activity cannot be attributed to one compound but rather requires a combination of the remedy’s components. Our results highlight this remedy has potential for the topical treatment for a number of *Candida* infections.
Echinocandin sensitivity profiles of *Candida glabrata* reveal different drug resistance mechanisms

Timothy Nickels, Kyle Cunningham

The Johns Hopkins University, USA

Abstract

*Candida glabrata* is naturally resistant to azole-class drugs, leaving echinocandin drugs as a more attractive treatment alternative for this fast-growing public health threat. However, *C. glabrata* is also acquiring resistance to echinocandins. While hotspot regions of the target FKS genes are the main drivers of resistance, there are other mechanisms that are largely uncharacterized. To better identify and define these mechanisms, we employed a transposon insertion mutagenesis approach to screen mutants resistant to varying doses of echinocandin drugs. Disruption of the FKS genes led to high echinocandin sensitivity, providing a validation for our approach. Insertion mutants in genes encoding N-linked glycosylation enzymes (ALG5, ALG6, ALG8) demonstrated high resistance in the three echinocandin drugs (micafungin, caspofungin, anidulafungin) as well as the new FKS inhibitor ibrexafungerp. Aside from this pathway, each produced very different resistance/sensitivity profiles, indicating variable mechanisms of resistance for each drug. This finding contrasts with previous assumptions of similarity between the different drugs in this class. The sphingolipid biosynthesis pathway contributes to caspofungin sensitivity, but not other echinocandins. Ergosterol biosynthesis contributes to anidulafungin sensitivity, and phospholipid acyltransferase SLC1 contributes to micafungin sensitivity. Lipid pathway genes exhibited little influence on ibrexafungerp. All together, our data provides deeper insight into the mechanisms of action for each of these drugs and points to potential targets for combination therapies to enhance clinical outcomes for cases of invasive candidiasis.
An inducible CRISPR interference system to study essential gene function in *Candida albicans*

Lauren Wensing¹, Meea Fogal¹, Abdul-Rahman Adamu Bukari², Anthony Hendriks¹, Nicholas Gervais¹, Clara Fikry¹, Aleeza Gerstein², Rebecca Shapiro¹

¹University of Guelph, Canada. ²University of Manitoba, Canada

Abstract

With the emergence of antifungal resistant *Candida albicans* strains, the need for new antifungal drugs is critical in combating this fungal pathogen. Investigating essential genes in *C. albicans* is a vital step in characterizing putative antifungal drug targets. As some of these essential genes are conserved between fungal organisms, developed therapies targeting these genes have the potential to be broad-range antifungals. In order to study these essential genes, classical genetic knockout or CRISPR-based approaches cannot be used as disrupting essential genes leads to lethality in the organism. Fortunately, a variation of the CRISPR system (CRISPR interference or CRISPRi) exists that enables precise transcriptional repression of the gene of interest without introducing genetic mutations. CRISPRi utilizes an endonuclease dead Cas9 protein which can be targeted to a precise location but lacks the ability to create a double-stranded break. The binding of the dCas9 protein to DNA prevents the binding of RNA polymerase to the promoter through steric hindrance. We recently published the novel use of this technology in *C. albicans* and are currently working on expanding this technology to large-scale repression of essential genes. Through the construction of an essential gene CRISPRi-sgRNA library, we can begin to study the function of essential genes under different conditions and identify genes that are involved in critical processes such as drug tolerance in antifungal resistant background strains. These genes can ultimately be characterized as putative targets for novel antifungal drug development, or targeted as a means to sensitize drug-resistant strains to antifungal treatment.
Regulation of *THI* gene expression in *Candida albicans*

Ronda Rolfes, Josh Tobin, Finn Thompson, Michael Chorabik

Georgetown University, USA

Abstract

*Candida albicans* is an opportunistic fungal pathogen that is responsible for significant morbidity and mortality. Grf10 is a conserved homeobox transcription factor that is hypothesized to coordinate filamentation with metabolic regulation, shown for purine biosynthesis, one-carbon metabolism, and maybe other pathways; the Grf10 ortholog in Schizosaccharomyces pombe is a regulator of *THI* genes. In other yeasts, Pdc2 regulates *THI* genes. The relationship between Pdc2, Grf10, and thiamine biosynthesis in *C. albicans* has not yet been evaluated. Thiamine pyrophosphate is a critical enzyme cofactor involved in central carbon metabolism. Our data show that Pdc2 but not Grf10 is important for expression of *THI* genes. Pdc2 is necessary for hyphal formation under filamentation inducing conditions. Using RNAseq we identified the set of target genes regulated by thiamine and by Pdc2. Starvation of a *pdc2Δ* mutant in medium lacking thiamine led to changes global changes in carbon utilization genes. On-going experiments are examining how Pdc2 is up-regulating expression of *THI* genes in response to thiamine levels.
Genetic determinism of sulfur utilization in Candida albicans and its role in fungal fitness

Anagha Menon C T1,2, Faiza Tebbji2, Antony Vincent3, Adnane Sellam1,2

1Université de Montréal, Canada. 2Institut de Cardiologie de Montréal, Canada. 3Université Laval, Canada

Abstract

Metabolic flexibility is an important virulence strategy in Candida albicans which enables the colonization of diverse host niches. Sulfur metabolic flexibility in C. albicans remains largely unexplored. Taurine is one of the abundant sulfur sources in the gut which may enable sulfonate and sulfur acquisition under sulfur scarcity. A recent study reported the relevance of taurine in regulating the gut micro-ecology, by inhibiting the growth of harmful bacteria. So far, little is known regarding the ability of C. albicans to utilize this abundant sulfur source and its contribution to fungal fitness. Our study shows that C. albicans utilize taurine as a sole source of sulfur but not as a nitrogen or carbon source. By protein homology analysis we identified candidate genes that could be responsible for taurine metabolism in C. albicans. We identified 5 orthologs of Jlp1 gene, an Fe(II)-dependent sulfonate/alpha-ketoglutarate dioxygenase, involved in sulfonate catabolism in S. cerevisiae. We also identified two transcriptional regulators of sulfur utilization, Cbf1 and Met32 believed to play a role in regulation of sulfur starvation and sulfur amino acid biosynthesis. The mutants of Cbf1 and Met32 impede taurine utilization. By transcriptomic analysis, we characterized the sulfur starvation and taurine utilization signatures and elucidated sulfur metabolic pathway in C. albicans. Different sulfur utilization mutants showed attenuated virulence when tested for their in vivo fitness using different infection models. Fungal sulfur metabolism being significantly distinct from humans, our study is aimed to give insights into potential targets for development of new therapeutic strategies.
Intrahost diversity of *Candida* species as invasive or commensal samples from single isolates or metagenomes

Hannah Snell¹, Terrance Shea¹, Daniel Floyd², Johnny Atallah², Michael Mansour², Christina Cuomo¹

¹Broad Institute of MIT and Harvard, USA. ²Massachusetts General Hospital, USA

Abstract

*Candida* species are members of the human microbiome, however people who are immunocompromised are at higher risk for developing candidiasis and invasive candidemia. We have little understanding of the evolution of infective *Candida* strains when at-risk patients present with these diseases in the clinical environment. We characterized the diversity of *Candida* present in patients with candidemia, comparing bloodstream isolates to those found at other body sites and reservoirs. The patient cohort included adult subjects with candidemia categorized among three different groups: individuals with leukemia/hematopoietic stem cell transplantation, individuals with indwelling catheters, and individuals with other reasons for candidemia. We carried out whole genome sequencing (WGS), whole metagenomic sequencing (WMS), and internal transcribed spacer (ITS) sequencing for cultured patient blood isolates, stool samples, and skin swabs, respectively. Most commonly *Candida albicans*, *Candida glabrata*, and *Candida parapsilosis* were detected, with additional cases of five other fungal species. We found that blood isolates (WGS) from the same patients are highly identical to each other and to *Candida* from stool (WMS), however in-depth comparisons of WGS blood and WMS stool samples revealed genetic variation unique to each sample or present at different frequencies in WMS data. In many cases, the primary species detected by ITS analysis from skin swabs also matched the WGS and WMS data. Our results support that a single *Candida* species often colonizes multiple body sites, with a connection between a single clonal strain in the gut and the blood, highlighting a short evolutionary path during the progression of infection.
Manganese homeostasis shapes fungal biology and virulence of *Candida albicans*

Manon Henry¹, Antony Vincent², Inès Khemiri¹, Faiza Tebbji³, Adnane Sellam¹,³

¹Université de Montréal, Canada. ²Université Laval, Canada. ³Institut de Cardiologie de Montréal, Canada

Abstract

Trace metals including iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) are essential micronutrients that are sequestered by the host to limit microbial growth, a process known as “nutritional immunity”. For *Candida albicans*, adaptation for acquisition of these metals is essential for its survival and its ability to infect the host. Many virulence factors such as the superoxide dismutases require these metals for their activity. While seminal contributions were made to understand how fungal pathogens acquire Fe, Cu and Zn, processes required for Mn internalization and keeping this central metal at homeostatic level remain completely unexplored. To understand the contribution of Mn homeostasis in *C. albicans* virulence, we performed RNA-seq transcriptional profiling under different Mn levels. Our data emphasized a significant impact of Mn fluctuations on different biological and virulence processes. We also uncovered that genetic inactivation of different Mn transporters (SMF12, SMF11, SMF2 and SMF3) members of the Nramp (Natural resistance-associated macrophage proteins) play a key role in Mn homeostasis, antifungal tolerance, unfolded protein response and host invasion. We finally performed in vivo experiments and found that *C. albicans* cells pre-grown in Mn-depleted medium caused more damage to human enterocytes as compared to cells grown under Mn sufficiency. To our knowledge, this work represents the first assessment of the genetic determinism of Mn homeostasis and its contribution to *C. albicans* virulence.
B166

Mechanism and inhibition of the \textit{C. albicans} microtubule length regulator \textit{CaKip3}

\textbf{Byron Hunter$^1$, Michelle Gontcharova$^1$, Andrea Petkovic$^1$, Matthieu Benoit$^2$, Corey Frazer$^{3,1}$, Kody Klupt$^1$, Ana Asenjo$^2$, Hernando Sosa$^2$, John Allingham$^1$}

$^1$Queen’s University, Canada. $^2$Albert Einstein College of Medicine, USA. $^3$Brown University, USA

Abstract

Microtubules are the main component of the mitotic spindle, which is responsible for the movement of nuclei and accurate segregation of chromosomes during cell division. We show that the kinesin-8 motor protein \textit{CaKip3} regulates these functions of the mitotic spindle in \textit{Candida albicans} by controlling microtubule polymer length. Accordingly, \textit{C. albicans} strains lacking \textit{CaKip3} display hyper-elongated spindles and undergo cell cycle arrest. Using \textit{in vitro} biochemical experiments, we show the \textit{CaKip3} motor domain acts as a motile microtubule depolymerase that travels to microtubule ends and catalyzes the removal of tubulin subunits. Our crystallographic and cryo-EM structures of the \textit{CaKip3} motor domain reveal the mechanistic basis for these activities and show that the mechanochemical cycle involved is unique from other kinesin motors. Importantly, we can specifically inhibit the biochemical activity of \textit{CaKip3} with a kinesin-8 inhibitor and show that treating \textit{C. albicans} cells with this inhibitor mirrors the \textit{CaKip3} knockout phenotype. Collectively, this research provides new insight into a mitotic spindle regulatory factor in \textit{C. albicans} and its potential utility as an anti-fungal target.
Evolutionary mechanisms governing biofilm network rewiring in the Candida species

Deepika Gunasekaran, Austin Perry, Jaspreet Kaur, David Ardell, Clarissa Nobile

University of California, Merced, USA

Abstract

Biofilm formation in Candida albicans is governed by a recently evolved transcriptional network consisting of nine transcription factors (TFs) and approximately 1000 target genes. Studies have shown that core elements of the biofilm network vary between and within Candida species. Here, we elucidate the mechanisms driving evolution of the C. albicans biofilm regulatory network. Protein coding genes and regulatory elements were compared across Candida species, and three non-Candida fungi as outgroups, to obtain conserved components of the biofilm network. Functional enrichment was performed to identify crucial steps in biofilm development that are conserved across large evolutionary distances. Our results indicate that downstream protein-coding target genes are highly conserved compared to TF-target interactions. Additionally, the conservation of target genes appears independent of the number of upstream regulators and the genes regulated by a single TF are as conserved as the genes regulated by all TFs. These findings suggest that although the overall structure of the network is conserved, its gene composition is not. Conservation of target genes was also found to be independent of upstream regulators. Interestingly, the targets of Ndt80 (a TF conserved across large evolutionary distances) are as conserved as targets of Rob1 (a newly emerged TF conserved only in C. albicans and its close relatives). Overall, we find that the mechanisms of regulatory network rewiring in the biofilm network are primarily through cis-regulatory modifications, followed by large scale duplications and modifications of TF functions.
Using the Auxin Inducible Degron (AID) system to validate the histone acetyltransferase, Gcn5, as an antifungal drug target in *C. glabrata*

Smriti Hoda¹, Justin Gregor¹, Bao Vu², Scott Moye-Rowley², Mark Hall¹, Scott Briggs¹

¹Purdue University, USA. ²University of Iowa, USA

Abstract

*Candida glabrata* is the second most common bloodstream *Candida* infection and is of critical concern because of its ability to develop azole and echinocandin drug resistance. Therefore, additional research and tools are needed to further understand the molecular mechanisms of antifungal drug resistance and to identify new antifungal drug targets. Epigenetic factors that modify histones such as Gcn5, a known histone H3 acetyltransferase, are important epigenetic regulators required for the adaptation to various cellular stresses. In addition, loss of *GCN5* in *C. glabrata* and *C. albicans* increases the antifungal drug susceptibility and decreases virulence, suggesting that Gcn5 and/or other epigenetic factors as potential antifungal drug targets. For testing factors that could serve as target antifungal drug targets, we established the auxin-inducible degradation (AID) and second generation AID2 system in *C. glabrata*. We show that AID-tagged Gcn5 is rapidly depleted upon auxin (IAA or 5-Adamantyl-IAA) treatment. *C. glabrata* that is depleted for Gcn5 show susceptibility to antifungal drugs and altered gene expression similar to a *gcn5Δ*. Overall, our data suggests that the selective and specific targeted degradation using the AID system makes it a useful platform for validating antifungal drug targets as well as a tool for studying mechanisms of drug resistance and pathogenesis.
Investigating the cellular responses of *Candida albicans* to echinocandins and the influence of natural variation in chitin content

Mark Peacock¹, Alessandra da Silva Dantas², Neil A.R. Gow³, Carol Munro⁴, Ian Leaves³

¹MRC centre medical University of Exeter, United Kingdom. ²University of Newcastle, United Kingdom. ³MRC centre medical mycology University of Exeter, United Kingdom. ⁴University of Aberdeen, United Kingdom

Abstract

Echinocandins are frontline antifungal drugs that inhibit cell wall β-1,3-glucan production by the β-glucan synthase complex. Resistance to echinocandins arises sporadically with point mutations in the FKS gene(s) resulting in an increased Minimal Inhibitory Concentration (MIC). Susceptible strains can exhibit echinocandin tolerance - a time dependant mechanism whereby a subpopulation of cells can grow slowly in the presence of drug. Susceptible subpopulations of *C. albicans* remain viable when exposed to supra-MIC levels of echinocandins - a phenomenon called “paradoxical growth”, which is mechanistically not well understood. Following exposure to supra-high MIC caspofungin (CSF), changes to cell wall architecture (increased chitin and exposed chitin) and yeast cell enlargement was observed. This was accompanied by G2 cell cycle arrest. By using cell sorting flow cytometry, we separated *C. albicans* cells according to their chitin content (low vs high). The population of high chitin cells were less susceptible to echinocandins and had a higher prevalence of antifungal-tolerant colonies. Furthermore, naturally occurring high chitin cells displayed the pronounced cellular enlargement that is associated with yeast survival in the presence of supra-MIC CSF. We therefore propose that there is a coordinated process that allows otherwise susceptible *C. albicans* cells to survive in the presence of CSF. This process starts with cellular enlargement of naturally occurring high chitin cells and further increase in chitin content and exposure.
Host-\textit{C. albicans} interactions during catheter-associated urinary tract infections

Alyssa La Bella, Felipe Santiago-Tirado, Ana Flores-Mireles
University of Notre Dame, USA

Abstract

Background: Candiduria (\textit{Candida} in urine) is one of the most problematic issues in patient management due to a lack of evidence-based information on \textit{Candida} pathogenicity in the urinary tract, as it is unclear whether candiduria results from contamination, commensalism, or invasive infection, representing a conundrum for the physician. Recently, \textit{Candida} spp—specifically \textit{Candida albicans}—are a major causative agent of catheter-associated urinary tract infections (CAUTIs), becoming the second most common CAUTI uropathogen. We found that the catheterized-bladder environment induces filamentation and an Efg1-dependent virulent programming in \textit{C. albicans}. However, it is unclear if, during fungal CAUTI, the bladder responds differently to fungal infection or commensal colonization. Hence, we focus on characterizing the host-fungal interaction that results in fungal CAUTI.

Methods: We used \textit{Candida albicans} SC5314 WT and yeast-locked strain (\textit{efg1Δ/Δ}) in our well-established CAUTI mouse model. Furthermore, bladder colonization, inflammatory, and immune response were analyzed by CFU enumeration, cytokine profiling, immunohistochemistry, immunofluorescence, and flow cytometry.

Results: We found that the WT strain, but not the yeast-locked strain \textit{efg1Δ/Δ}, was able to cause CAUTI, successfully colonizing the bladder and catheter at 24hpi. We also found differential inflammatory and immune response to fungal morphology, where WT strain induced an exacerbated bladder inflammation, inflammatory cytokines induction, and increased neutrophils recruitment and NETosis activation while the yeast-locked strain response was similar to catheterized bladders that were non-infected.

Conclusion: These suggest that the catheterized-bladder environment induces \textit{Candida} hyphal morphology and the bladder responds with exacerbated inflammation and neutrophil recruitment to control fungal infection.
Optimizing and Scaling up CRISPR interference for pooled genome-wide functional genomics in *Candida albicans*

Meea Fogal, Lauren Wensing, Nicholas Gervais, Rebecca Shapiro

University of Guelph, Canada

Abstract

*Candida albicans* is an opportunistic pathogen and a commensal member of the human microbiota. Systemic infections caused by *C. albicans* can be life-threatening and in many instances originate from the gastrointestinal tract. The fundamental molecular mechanisms that underpin both commensalism and pathogenicity in *C. albicans* are poorly understood. Genetic manipulation is critical to understand such molecular mechanisms. The Shapiro lab has validated the use of a transcriptional repression system, known as CRISPR interference (CRISPRi), to probe gene function in *C. albicans*. In optimizing our CRISPRi system, we have identified a region of target for our CRISPRi system relative to the transcriptional start site (-200 bp to +50 bp). We have also validated that the system can be regulated via tetracycline and is able to repress the transcription of even essential genes. We have scaled this system up to generate a pooled CRISPRi plasmid library targeting 5953/6193 (96%) ORFs in the *C. albicans* genome. We aim to transform this plasmid library into the fungus to generate the first inducible, pooled CRISPRi functional genomic library in *C. albicans*. Creating this pooled genome-wide library will allow us to rapidly screen the genome for genes important to survival in physiologically relevant conditions via an *in vitro* model of the human gut, host-pathogen interactions, and antifungal drug susceptibility.
Within-patient genomic and phenotypic diversity of *Candida glabrata* (CG) from positive blood cultures (BCs) of patients with bloodstream infections (BSIs)

Hassan Badrane¹, Shaoji Cheng¹, Christopher Dupont², Binghua Hao³, Anthony Newbrough¹, Giuseppe Fleres¹, Drishti Kauls², Cornelius Clancy¹,⁴, Hong Nguyen¹

¹University of Pittsburgh School of Medicine, USA. ²JCVI, USA. ³UPMC, USA. ⁴V.A. Pittsburgh Healthcare System, USA

Abstract

Infections due to CG are associated with high mortality. CG manifests striking genomic plasticity, which is believed to facilitate adaptation and survival under diverse host conditions. Population studies of CG within the same host are limited. Here we studied up to 10 unique colony from BC bottles of 10 patients with CG BSI, using short- and long-read whole genome sequencing. Analysis of within-patient strains showed a high degree of genetic variability with SNPs and indels. The 94 clinical isolates encompassed 3 distinct clades in the genome phylogeny. Each patient was infected with strains that clustered among themselves. Genetically diverse strains from two patients underwent detailed phenotypic testing. In both patients, strains exhibited differences in antifungal resistance and susceptibility to phagocytosis. BCs were comprised of mixed fluconazole-susceptible and resistant-populations, although the clinical lab did not identify fluconazole-resistant clones. In one patient, fluconazole-resistant clones displayed respiratory-deficient small colony variant (SCV) phenotype, and had highly variable mitochondrial genome mutations. SCVs had numerous mutations or low copy numbers of mitochondrial genomes. This patient subsequently succumbed to infection by fluconazole-resistant SCV strain. The second patient harbored a mixed population of strains with wild-type (fluconazole-susceptible) and gain-of-function Gly346Cys Pdr1 mutation (fluconazole-resistant). Our data challenge the long-standing, “single organism” model of pathogenesis, and suggest a population-based paradigm of CG genetic and phenotypic diversity during BSI. Results have potential implications for medical and clinical microbiology practices and for understanding emergence of CG antifungal resistance, treatment responses, pathogenesis and adaptation.
TAC1B-mediated fluconazole resistance in Candida auris is driven by overexpression of CDR1

Jeffrey Rybak¹, Katherine Barker¹, Christina Cuomo², David Rogers¹

¹St. Jude Children's Research Hospital, USA. ²Broad Institute, USA

Abstract

Previously we have shown that mutations in TAC1B are potent contributors to clinical fluconazole (FLC) resistance in Candida auris, named by the CDC and WHO as an urgent threat to public health. In the present study, we examined the most prevalent TAC1B mutations associated with triazole resistance: A640V (clade Ic), A657V (clade Ib), and N773_L774del (clade IV; referred to as “ADdel”). Each TAC1B mutation was introduced individually by CRISPR-Cas9 mediated gene editing into an isogenic FLC-susceptible strain (TAC1B-WT) with subsequent gene expression profiling by RNAseq. When each derivative strain was compared to TAC1B-WT for genes differentially expressed at least 1.5-fold, sixteen (eight up-regulated), 880 (530 up-regulated), and 98 (71 up-regulated) genes were in the A640V, A657V, and ADdel strain data sets, respectively. Moreover, thirteen genes were commonly differentially expressed and included CDR1. Quantitative RT-PCR revealed CDR1 gene expression was increased approximately 2- to 7-fold in the TAC1B mutant strains compared to TAC1B-WT and that CDR1 expression could be induced further in all strains upon 6-hr. treatment with 16 mg/L FLC. FLC MICs were subsequently measured in all strains: TAC1B-WT= 2 mg/L, TAC1B-A640V= 32 mg/L, TAC1B-A657V= 64 mg/L, and TAC1B ADdel= 64 mg/L, and disruption of CDR1 in these strains led to stark reduction in FLC MIC in each strain: TAC1B-WT= 0.125 mg/L, TAC1B-A640V= 0.5 mg/L, TAC1B-ADdel= 0.5 mg/L. In contrast to previous studies, these results indicate that CDR1 has a direct, significant role in TAC1B-mediated fluconazole resistance in C. auris.
The ZCF27 transcription factor alters drug susceptibility and azole-induced gene expression in *Candida glabrata*.

DEBASMITA SAHA¹, Scott Briggs²

¹PURDUE UNIVERSITY, USA. ²Purdue University, USA

Abstract

*Candida glabrata* is naturally resistant to azole antifungal drug but also can become clinically resistant to this class when treating a *Candida* infection. Understanding the mechanisms of antifungal drug resistance and identifying factors contributing to drug resistance is greatly needed. Zinc cluster transcription factors such as Pdr1 and Upc2 are known to play key roles in azole drug resistance by controlling the expression of ABC transporters or ERG gene expression. The principal transcription factor controlling azole-induced *ERG11* expression in *C. glabrata* is Upc2a. However, it is unclear if other transcription factors contribute to the azole-induced expression of *ERG11* and/or other genes in the ergosterol pathway. Here we show that deletion of *HAP1* in *Saccharomyces cerevisiae* or deletion of ZCF27 in *C. glabrata*, the closest known ortholog to *S. cerevisiae* Hap1, results in azole susceptibility while supplementation with exogenous ergosterol can suppress the azole susceptibility of zcf27Δ. Furthermore, gene expression analysis revealed that azole-induced transcript levels of ERG genes, including the azole target, *ERG11*, are significantly reduced in the zcf27Δ strain. Overall, we have identified another ZCF transcription factor that contributes to ergosterol homeostasis under azole treatment.
Regulation of Phosphatidylinositol-(4,5)-bisphosphate and active-Rho1p Levels and distribution is crucial for correct spatio-temporal cytokinesis in Candida albicans

Hassan Badrane1, Hong Nguyen1, Cornelius Clancy1,2

1University of Pittsburgh School of Medicine, USA. 2V.A. Pittsburgh Healthcare System, USA

Abstract

Background: We previously shown that the EH domain-containing protein Irs4p binds the 5-phosphatase enzyme Inp51p to regulate the intracellular levels of Phosphatidylinositol-(4,5)-bisphosphate (PIP2) in C. albicans. Deletion of the gene encoding either of the proteins led to elevated levels of PIP2 and presence of abnormal intracellular membranous PIP2 patches. Our overall results merged toward an interplay between PIP2 and septins to regulate PKC-Mkc1 cell wall integrity pathway, echinocandin and cell wall stress responses, and virulence during candidiasis. Our goal was to define the effect of disrupting PIP2-septin pathway interactions.

Methods: We created various CaGFP- and CaRFP-tagged reporters and used live cell imaging to localize pathway components. We also relatively quantified active-Rho1p using western blot.

Results: By following the appearance of these abnormal PIP2 patches, we found that they highly correlate in space and time with the last step of cytokinesis which separates mother and daughter cells. The vast majority of PIP2 patches arise during or after this step, and very close to the division site. Further, these abnormal PIP2 patches colocalize with the two main actomyosin components (Act1p and Myo1p) and the active form of Rho1p which functions in different phases of cytokinesis including the rearrangement of actin cytoskeleton. Finally, we show that strikingly similar but distinct defects are caused by exposure of wild-type cells to caspofungin.

Conclusions: Our data support a model in which echinocandins and other stressors activate PKC-Rho1, resulting in PIP2-septin interactions that recruit actinomyosin and regulate cell wall integrity responses, septation and cytokinesis.
Structural analysis of Als adhesins from *Candida albicans*

Andrea Petkovic, Byron Hunter, John Allingham

Queen's University, Canada

Abstract

*Candida albicans* colonizes its host by adhering to epithelial cells or medical devices and by forming biofilms. Adhesion of *C. albicans* to these biotic and abiotic surfaces is primarily dictated by the agglutinin-like sequence (Als) adhesins, a family of cell-wall-associated glycoproteins that contain a highly-conserved protein-binding cavity at their N-terminus, a central 36-amino-acid tandem repeat region, and a Ser/Thr/Asn-rich C-terminal domain that attaches the protein to the fungal cell wall via the remnant of a GPI anchor. Recent studies have shown that adhesion of *C. albicans* to surfaces is enabled by shear force-induced formation of linear aggregates, or amyloids, of Als adhesins. A conserved amyloid forming region (AFR) in a few Als adhesins have been implicated in this aggregation and have been suggested to be responsible for the robustness of *Candida* biofilms. It is hypothesized that when Als proteins are not bound to a host surface, their AFR is concealed by its flanking regions. However, we do not yet know what structural changes need to occur in Als proteins to expose the AFR and produce amyloid formation. Using experimental and *in silico* structural biology approaches along with biophysical analyses of purified wild type and mutant *C. albicans* Als proteins, we will identify residues flanking the AFR that regulate its accessibility for Als protein amyloidogenesis.
A prebiotic-based diet modulates the oral microbiome composition, attenuating oropharyngeal candidiasis in mice

Roberto Vazquez-Munoz, Angela Thompson, Takanori Sobue, Anna Dongari-Bagtzoglou
UConn Health, USA

Abstract

The oral microbiome can influence the ability of Candida albicans to cause oropharyngeal candidiasis (OPC). We recently reported that a Lactobacillus johnsonii-enriched oral microbiota reduced C. albicans virulence in mice and that the dominant mouse strain MT4 has anticandidal properties. Prebiotic Xylo-OligoSaccharides (XOS) increase gut lactobacilli, therefore, we evaluated the impact of XOS (3-week diet, w/wo MT4) in the oral microbiome of C57BL/6 female mice and Candida virulence. We assessed changes in the oral microbiome composition via 16S-rRNA gene high throughput sequencing, validated by qPCR and culture methods. XOS impact in Candida infection (OPC mouse model) was assessed by changes in oral fungal and bacterial biomass and scoring tongue lesions.

XOS modulated the oral microbiome composition, increasing Bifidobacterium abundance -displacing Lactobacillus-, and attenuating the increase of OPC-associated enterococci and staphylococci. MT4 supplementation post-XOS diet increased lactobacilli abundance, further reduced OPC-associated bacteria, and partially restored the basal oral microbiome composition. In the OPC model, XOS attenuated the tongue lesions and bacterial dysbiosis. In conclusion, XOS had a protective effect against OPC, possibly by modulating the oral microbiome towards a bacterial structure more resilient to Candida infection. MT4 partially restored the oral bacterial communities, suggesting it plays a regulatory role in community structure and composition. As MT4 is closely related to the probiotic L. johnsonii NCC533, further research on its probiotic potential is of clinical interest. This is the first time, to our knowledge, that lactobacilli and XOS modulatory activity on the oral microbiome, and XOS protective effect against OPC, are reported.
Alternative oxidase confers tolerance to high iron-mediated oxidative stress in *Candida albicans*

Rishabh Sharma¹, Andrew Gibb², John Elrod², Sumant Puri¹

¹Temple University, Oral Health Sciences, USA. ²Temple University, Center for Translational Medicine, USA

Abstract

Background: *C. albicans* alternative oxidase (Aox) removes excess electrons to shield the mitochondria from respiratory stress. Here, we assess the role of Aox in protecting mitochondria from high iron-mediated reactive oxygen species (ROS).

Methods: *C. albicans* wild-type (WT) growth was assessed in 0.5-500 μM of iron. Intracellular iron, labile iron, ROS level, and oxygen-consumption-rate (OCR) were evaluated using ICP-MS, Calcein-AM, DCFH-DA staining, and Seahorse-analyzer, respectively. Antioxidant genes expression were measured by qPCR in high and low iron. Mitochondrial-ROS was compared between WT and Aox null mutant (*aox1-A/aox1-A aox1-B/aox1-B*) under high and low iron. Oropharyngeal candidiasis (OPC) model with low and high iron mice was used to assess fungal-burden in tongue-tissue of mice infected with WT and *aox1-A/aox1-A aox1-B/aox1-B* cells.

Results: *C. albicans* showed a pronounced flexibility to iron availability, with optimal growth from 1-500 μM. Compared to 1 μM, high iron (500 μM) showed a 1237.98-fold increase in intracellular iron, a 155% increase in the labile-iron pool, and a 4.45-fold increase in ROS-levels; yet high iron cells showed a 2.5-fold increase in ATP. OCR was also found to be significantly increased (62.8%) in high iron, with a greater contribution of Aox to OCR under high iron. High iron-induced cyclical expression of AOX contributed towards mitigation of mitochondrial ROS. High iron mice infected with *aox1-A/aox1-A aox1-B/aox1-B* cells showed significant reduction in tongue fungal burden.

Conclusion: *C. albicans* exhibits high iron-tolerability. Aox is crucial for this by preserving mitochondrial function under high iron.
Cellular and extracellular vesicle response of *Candida auris* to caspofungin treatment

Isadora Rocha¹, Haroldo Oliveira¹, Rafaela Amatuzzi¹, Daniel Zamith-Miranda², Joshua Nosanchuck², Lysangela Alves¹

¹Fundação Oswaldo Cruz - Fiocruz, Brazil. ²Albert Einstein College of Medicine, USA

Abstract

The emerging yeast *Candida auris* causes bloodstream infections with mortality rates varying up to 78%. The strains are intrinsically multiresistant to both antifungals – including some pan-resistant strains - and sanitizing agents. Health organizations have been putting efforts into understanding the specie’s biology and how it interacts with the host's immune system. Our studies characterize the response of *C. auris* to caspofungin treatment, the recommended antifungal drug for the species, as well as its extracellular vesicle (EV) population as a part of the organisms, counteract to antifungal agents. Our transcriptomic experiments reveal a change in both the cellular and EV content of RNAs, supported by the proteomics results of the same dataset, relating to a shift in cell wall composition, ribosome synthesis and regulation, cell cycle, and stress response. Moreover, the study of how the caspofungin treatment affects the vesicles’ interaction with the immune system (Illustrated by dendritic activation) and endothelial cell transcriptomic response highlights the potential of EVs as a disease-enhancing factor. Our work has been adding new information on how *C. auris* and the host respond to antifungal treatment, its survival mechanisms leading to resistance, and serving as the basis for studies of how RNA molecules are directed to EVs upon infection and caspofungin contact.
Set1-mediated histone H3K4 methylation alters ergosterol homeostasis, antifungal drug susceptibility and pathogenesis of *Candida glabrata*.

Kotany Baker, Smriti Hoda, Debasmita Saha, Justin Gregor, Scott Briggs

Purdue University, USA

Abstract

*Candida glabrata* is the second most commonly isolated species from *Candida* infections, coming in second to *C. albicans*. Treatment of *C. glabrata* infections are difficult due to their natural resistance to antifungal azole drugs and their ability to adapt and become multidrug resistant. However, the role of epigenetic histone modifications in azole-induced gene expression and drug resistance is poorly understood in *C. glabrata*. In this study, we investigated the contributing cellular factors for controlling drug susceptibility where we have determined that Set1, a Histone H3K4 methyltransferase, is necessary for the expression of genes involved in the ergosterol biosynthesis pathway, an essential pathway that antifungal drugs target. This epigenetic mechanism also is needed to maintain ergosterol homeostasis under azole treatment and WT azole drug susceptibility. In addition, *C. glabrata* lacking *SET1* shows reduced virulence as determined by a *Galleria mellonella* larvae infection model and altered *in vivo* drug efficacy. Overall, our data suggests that Set1 is a viable antifungal drug target candidate that has the potential to alter fungal pathogenesis and/or reclaim the utility of existing antifungal drugs against drug resistant fungi.
Antifungal vaccine: An alternative approach in combatting antifungal resistance

Aijaz Ahmad

University of the Witwatersrand, South Africa. National Health Laboratory Service, South Africa

Abstract

The optimism that the miracle drug “penicillin”, and the other drugs developed during the golden age of antibiotic discovery, would drive infectious diseases to extinction was unfortunately unfounded. In 2023, we find ourselves facing the global threat of a post-antibiotic era. Current decreasing effectiveness of antibiotics in general and antifungals in particular has increased drastically. Not only the new drugs but also novel strategies such as vaccine development to combat antifungal resistance are of immediate priority.

Cell wall proteins in Candida species are responsible for eliciting immune response in hosts and therefore can be promising candidates for the development of antifungal vaccines against Candida. In present study, the cell wall proteome of C. auris was characterized by using LC-MS followed by bioinformatic and functional analyses. Furthermore, the protective effect of the cell wall associated protein against C. auris infection was evaluated in BALB/c female mice. A total of 60 proteins were predicted to be immunodominant. In vivo results demonstrated lowered fungal burden in target organs and increased survival in vaccinated mice as compared to the infection control group revealing the immunogenic property of cell wall associated proteins. Therefore, this study validates the mass-spectrometry approach to identify antigenic proteins of C. auris and the potential of these proteins to serve as biomarkers for development of diagnostic assay and/or vaccines against infections caused by C. auris.
Identifying genetic regulators of dispersion in Candida albicans biofilms

Stephanie Geller, Megan McClean
University of Wisconsin-Madison, USA

Abstract

Candida albicans is an opportunistic fungal pathogen that causes systemic infection in immunocompromised individuals by establishing biofilms on medical devices such as catheters. Systemic infections can develop by Candida yeast cells adhering to a surface, such as a medical device, and developing a biofilm which releases “dispersed” cells into the bloodstream. Candida strains deficient in dispersion have been shown to be less virulent, suggesting that dispersion plays an important role in disease progression. Therefore identifying additional genes in the pathway to dispersion could be important for halting the progression of infection. In a search for genes that are involved in dispersion, we screened a library of 165 transcription factor mutants in a 96 well plate assay for mutants that have higher or lower dispersive capacities than a wildtype biofilm. Using an improved 96 well assay/xtt protocol as well as an under-oil droplet screening assay we identified mutants that have higher dispersive capacities than wildtype biofilms while still having hyphal biofilm phenotypes. We selected one mutant (rob1) and wildtype to undergo RNA sequencing to tease out the gene expression differences throughout biofilm growth in the same strain as well as differences in expression between strains. From this we are investigating the role of genes controlled by rob1 and their relation to dispersion using additional optogenetic and under-oil tools.
The glories and shadows of mixed-species biofilms formed by \textit{C. albicans} and \textit{P. gingivalis}.

Ewelina Wronowska, Magdalena Surowiec, Miriam Gonzalez-Gonzalez, Magdalena Juszczak, Justyna Karkowska-Kuleta, Marcin Zawrotniak, Maria Rapala-Kozik

Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow, Poland

Abstract

\textit{Candida albicans}, which colonizes the human body, creates a polymicrobial biofilm with bacteria it encounters, which is challenging to eliminate. One of the bacteria that interact with \textit{C. albicans} is \textit{Porphyromonas gingivalis}, which prefers anaerobic conditions and is a predominant pathogen of periodontitis. Its main weapons destroying host tissues are proteolytic enzymes known as gingipains.

The aim of the presented research is to investigate the mechanisms involved in the mutual interaction between these microorganisms. Microscopic analyses with an oxygen fluorescence probe revealed that in normoxia, substantial oxygen consumption within the biofilm was observed due to the intensive growth of \textit{C. albicans} cells, which increases the survival of \textit{P. gingivalis}. Interestingly, the gingipains produced by bacteria during biofilm formation activate \textit{C. albicans}' growth, which was not observed for mutants lacking the ability to produce these enzymes. The presence of serum enhances their activities. Proteomic analysis of the mixed biofilm matrix indicated an increasing content of fungal proteins associated with carbohydrate metabolism. But the ability of \textit{C. albicans} to form filamentous cells was of secondary importance.

The increased activity of gingipains within the mixed biofilm influenced the surfaces of fungal cells, reducing their resistance to levofloxacin and amphotericin B, and disarmed epithelial cells that lose proper interleukin production and activated apoptosis. Under these conditions, the bacteria remained unrecognized by the host, and antibiotic therapy was ineffective. The complex mechanisms behind biofilms make them a serious threat to human health.

This work was financially supported by the National Science Centre of Poland (grant no. 2019/33/B/NZ6/02284).
Characterizing the role of the putative phosphatidylserine synthase in the pathogenicity of *Candida glabrata*

Jemma McLeish, Todd Reynolds

University of Tennessee, Knoxville, USA

Abstract

*Candida glabrata* is a ubiquitous fungus that contributes to approximately 25,000 cases of blood infections per year and show a significant increased resistance to echinocandins and azoles. This increased resistance has created an urgent need for finding new and improved treatment strategies. Several reports have revealed that phosphatidylserine (PS) synthase (Cho1) is essential for virulence in clinically relevant fungi such as *C. albicans* and *Cryptococcus neoformans*. Moreover, these fungi and other yeast have shown a variety of phenotypes upon loss of PS, including altered viability, defective cell wall structure, and perturbations to membrane composition. Currently, there is a paucity of data reporting on the role of the putative phosphatidylserine synthase (CgCho1) in the general biology and pathogenicity of *C. glabrata*. Therefore, to characterize this enzyme, we are generating a mutant to assess its enzymatic activity using a PS synthase assay, establish its impact on phospholipid profiles by mass spectrometry and characterize the role of PS in cell wall biology and cell growth phenotypes. Additionally, we will assess the impact of CgCho1 on virulence using a murine systemic candidiasis model. Preliminary attempts to disrupt *CgCHO1* suggest the gene may be essential to this organism, which differs from other ascomycetes such as *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *C. albicans*. The findings will be of interest to provide evidence that determines whether targeting PS synthesis is a potential treatment strategy for *C. glabrata*. 
Intravital imaging of the host-pathogen interaction in larval zebrafish to identify C. albicans immune evasion factors.

Bailey Blair
University of Maine, USA

Abstract

In 2020 Candida was the most frequent cause of bloodstream infections in the U.S. The first line of defense against these infections is the innate immune system. Previous work suggests that early immune response is critical in controlling C. albicans infection. However, it has been seen that C. albicans has strategies to evade the host immune system. Evidence suggests that the ability to transition from yeast to hyphal growth may facilitate immune evasion by limiting early phagocyte recruitment and uptake of C. albicans. Reduced containment of C. albicans can lead to uncontrolled hyphal growth, causing damage that can lead to death. However, the mechanism by which C. albicans limits recruitment or containment is unknown. To uncover factors important in innate immune evasion, we utilized the transparent larval zebrafish infection model to screen C. albicans mutants for altered virulence and immune response. Ten of the 130 mutants screened had markedly reduced virulence. Many of these mutants also induced an altered immune response. RIM101 and NMD5 were found to play a role in limiting phagocytosis, while CHT2, CEK1 and RBT1 were found to limit the recruitment of macrophages and or neutrophils to the infection site. These mutants will be useful in identifying fungal pathways that down regulate the different steps of the innate immune response during infection. These results highlight the ability of C. albicans to use multiple strategies that allow it to impair the immune response.
Genetic ablation of *Candida auris UPC2* confers rapid fungicidal activity to the triazoles and increased resistance to amphotericin B

Sarah J. Jones, Jeffrey M. Rybak
St. Jude Children's Research Hospital, USA

Abstract

Previously, genetic ablation of the transcription factor genes UPC2 and UPC2A has been shown to result in enhanced triazole susceptibility in *Candida albicans* and *Candida glabrata*, respectively. To determine the potential impact of the *Candida auris* UPC2 ortholog (B9J08 000270; CAU UPC2) on antifungal susceptibility, we disrupted CAU UPC2 in a pan-antifungal resistant C. auris clinical isolate (Kw2999-18) generating the strain KwΔupc2. We then measured minimum inhibitory concentrations (MIC) for clinically available antifungals using CLSI methodology, assessed fungicidal activity over 48 hours by time-kill assay using pharmacologically-relevant concentrations of AMB or posaconazole, and performed transcriptional profiling (RNAseq) following posaconazole treatment. MIC testing revealed KwΔupc2 exhibits an 8 to 16-fold decrease in MIC for all triazoles, a 6-fold increase in AMB MIC, and no change in echinocandin susceptibility relative to Kw2999-18. Time-kill studies revealed an overall increase (>2-log₁₀) in colony forming units per mL (CFU/mL) for KwΔupc2 following AMB (2mg/L) treatment. Conversely, treatment of KwΔupc2 with 0.25, 1, or 4mg/L of posaconazole resulted in fungicidal activity (>3-log₁₀ CFU/mL reduction from baseline) in as little as 4 hours. By comparison minimal change (<1-log₁₀) in CFU/mL was observed with Kw2999-18 following all treatments. RNAseq analysis revealed 833 genes differentially expressed (>2-fold change and FDR <0.01) between KwΔupc2 and Kw2999-18 following posaconazole treatment. Among genes most downregulated (>25-fold decrease) were C. auris ERG1, ERG2, ERG3, ERG6 and MDR1. Further characterization of the CAU UPC2 transcriptional network and components of this network key to unlocking the observed fungicidal activity of the triazoles is needed.
The S639F mutation in *Candida auris* FKS1 confers increased relative abundance of beta-glucans and resistance to rezafungin and clinically available echinocandins

Jeffrey M. Rybak

St. Jude Children’s Research Hospital, USA

Abstract

The echinocandins are relied upon as front-line therapy for invasive *Candida auris* infections, and resistance to the echinocandins represents a significant threat to patients with *C. auris* infections. We sought to determine the direct contribution of one of the most commonly reported mutations associated with echinocandin resistance (*FKS1*\textsuperscript{S639F}; encoding the S639F substitution) on *C. auris* echinocandin susceptibility and on the relative abundance of the major cell wall constituents beta-glucan and chitin. Our *C. auris*-optimized Episomal Plasmid Induced Cas9 (EPIC)-gene editing system was used to both introduce the *FKS1*\textsuperscript{S639F} mutation into the Clade III echinocandin-susceptible clinical isolate RVA001 (yielding strain RVA-FKS1\textsuperscript{S639F}), and to correct the *FKS1*\textsuperscript{S639F} mutation in a pan-antifungal resistant Clade Ic clinical isolate Kw2999-18 (yielding strain Kw-FKS1\textsuperscript{WT}). Rezafungin, micafungin, caspofungin, and anidulafungin minimum inhibitory concentrations (MIC) were determined by broth microdilution. Relative abundance of beta-glucan and chitin were assessed following growth to exponential phase in YPD broth by measuring fluorescence following aniline blue and calcofluor white staining, respectively. Introduction of the *FKS1*\textsuperscript{S639F} mutation in RVA001 resulted in dramatic increases in echinocandin MIC exceeding the current CLSI breakpoints (rezafungin) and epidemiologic cutoff values (micafungin, caspofungin, and anidulafungin). Conversely, correction of the S639F-encoding mutation in Kw2999-18 restored wild-type susceptibility to all echinocandins. Relative to RVA001, RVA-FKS1\textsuperscript{S639F} exhibited a 30 and 20% increase in beta-glucan and chitin abundance, respectively. While only beta-glucan abundance decreased (50%) in Kw-FKS1\textsuperscript{WT} compared to the parental Kw2999-18. Further characterization of the impact of *FKS1* mutations on *C. auris* antifungal susceptibility and cell wall composition is needed.
Characterization of Genetic Pathways Involved in the Function of a Novel Antifungal Peptide

Kayla Haberman, David McNabb, Ines Pinto

University of Arkansas, USA

Abstract

There is an urgent need for novel treatments for Candida infections. The utility of antimicrobial peptides for antifungal therapy has garnered significant interest. One promising family of peptides is the Histatins; naturally occurring peptides secreted into the oral cavity that display antimicrobial activity. Histatin 5 is a twenty-four amino acid peptide with strong antifungal activity. Studies from our laboratory identified a small histatin-derived peptide, KM29 (Y-K-R-K-F-K-R-K-Y), that yields greater fungicidal activity than Histatin 5 against multiple Candida species. We are focused on understanding the mechanism of action of KM29. Taking advantage of the sensitivity of Saccharomyces cerevisiae to the peptide, a genetic screen of the S. cerevisiae whole-genome deletion library was carried out to identify single gene-mutations that confer increased resistance or sensitivity to KM29. Once validated, the goal is to identify the homologous genes in Candida sp., mutate them and test their effect on KM29 activity. Predominant groups included genes involved in mitochondrial function and plasma membrane structure and transporters. All mutants further characterized were confirmed for resistance to the peptide by microdilution assays. Peptide uptake and intracellular localization was determined using a fluorescence tagged KM29-FITC (DOI: 10.1128/AAC.01698-19). We have started using CRISPR to mutate homolog genes in C. glabrata and test their susceptibility to KM29. We show that mutations in AGP2, a gene involved in polyamine transport, increase resistance to KM29. These studies may serve as the basis for understanding the general mechanism of action of KM29 as well as other small cationic antifungal peptides.
Impact of susceptibility testing method and genetic clade on the detection of amphotericin B resistance among Candida auris clinical isolates

Sarah J. Jones, Gregory A. Phelps, Richard E. Lee, Jeffrey M. Rybak
St. Jude Children's Research Hospital, USA

Abstract

Applying tentative Centers for Disease Control and Prevention (CDC) breakpoints, 30 to 50% of Candida auris clinical isolates are considered amphotericin B (AMB) resistant. While these breakpoints were derived using a murine model of candidiasis and minimum inhibitory concentrations (MIC) measured by bioMerieux diffusion strip, other methods of AMB MIC determination are commonly employed clinically. The significance of AMB susceptibility testing method for the detection of clinical AMB resistance in C. auris remains unknown. Forty C. auris isolates representing all known genetic clades were used for these studies. MIC for all clinically available antifungals were determined by broth-microdilution (BMD) using CLSI methodology, and AMB MIC were additionally assessed using diffusion test strips from two manufactures (bioMerieux [BMX] and Liofilchem [LFC]) applying CLSI methodologies with modifications as recommended by the CDC. Four (10%), thirteen (32.5%) and one (0.3%) isolate were determined to be AMB-resistant when using BMD, BMX, and LFC testing methods, respectively. The single isolate which considered AMB-resistant by all methods is known to harbor a mutation in ERG6. Notably, MIC determined by BMX were generally higher than BMD MIC for Clade I isolates, but lower than BMD MIC for isolates from other clades. MIC determined by LFC were typically lower than other methods. These data demonstrate the impact of AMB susceptibility testing method on determination of AMB resistance. Considering the limited number of treatments available for C. auris infections, it is essential that the AMB MIC testing method which best predicts clinical outcomes be identified and testing methods standardized.
B190

The creation of a high-throughput platform for the identification of antifungal and antivirulence compounds that target *Candida albicans*.

**Jehoshua Sharma, Rebecca Shapiro**  
University of Guelph, Canada

**Abstract**

*Candida albicans*’ transition from a human commensal to pathogen is often signalled by a change in the ovoid cell shape to a multicellular filamentous hyphae through morphogenesis. This switch enables invasive tissue and bloodstream infections, with mortality rates and a decrease in susceptibility to antifungals. Additionally the rise of antifungal resistant *C. albicans* isolates continue to result in globally decreasing available treatment options for these infections. The discovery of novel antifungals that remain effective against drug resistant strains remains an urgent clinical need. However, the high selection pressure these antifungals impose enabled the rise of antifungal resistance in the first place. A solution is to find compounds that can rescue the host from infection while maintaining a low selection pressure to decrease the evolution of resistance. One example of this would be an antivirulence compound that targets and inhibits virulence factors, such as *C. albicans*’ hyphal formation via morphogenesis.

My research then involved screening the supernatants of over 700 microbes isolated from either soil, plants or gut commensals to discover antifungal and antivirulence compounds with potent anti-*Candida* activity. The screen was conducted using *C. albicans* grown in the cell-free supernatants of these microbes and then inhibition of growth was measured by OD after 24 hours and hyphal inhibition using 384-whole-well microscopy.

My presentation will describe the initial optimization of this platform against the 700 microbes screened; the novel species identified with anti-*Candida* activity; and preliminary growth assays that characterise the successful hits as either possessing antifungal or antivirulence activity.
The role of transcription factor, Ofi1 in the regulation of white-opaque switching and filamentation in *Candida albicans*

Hananeh Ghafelehbashi¹, Manjari Shrivastava², Malcolm Whiteway¹

¹Concordia university, Montreal, QC., Canada. ²University of Montreal, Montreal, QC., Canada

Abstract

*Candida albicans* is a fungal pathogen that can cause infections in immunocompromised individuals. It can adapt to the host organism through different cellular shape transitions, such as filamentous growth that plays a critical role in the fungus's virulence and white to opaque switching which play a role in mating. *C. albicans* is diploid and typically heterozygous at the central regulator locus, *MTL*, controlling mating type. In addition to homozygosity of *MTL* genotypes, white-opaque switching is necessary for mating. White and opaque cells are morphologically and genetically distinct, and the difference in gene expression is responsible for the change in their appearance. The adaptation of *C. albicans* to host niches relies on transcriptional regulatory programs that can be controlled by complex networks of transcription factors (TFs). These factors can influence the cell's phenotypic behaviour and stability by acting through DNA binding sites or interlocking feedback loops. Zinc finger cluster transcription factors are a well-known fungal TF family. A novel transcriptional regulator, Ofi1, has been identified as a candidate regulator for both white-opaque switching and filamentation in *C. albicans*. In white-opaque switching it appears to act downstream of the master regulator Wor1. A comparison of the *ofi1Δ/ofi1Δ* strain, the Ofi1 activated strain and the wild type (SN148aa) strain under different carbon sources and temperatures were assessed. Activation leads to an increase in both processes, while deletion reduces the frequency of white-opaque switching. The aim of the project is to investigate the role of Ofi1 in the transcriptional programs involved in these processes.
**A192**

*Candida albicans* (Ca) from bloodstream infections (BSIs) demonstrate within-patient diversity in genotypes and phenotypes, including *in vitro* and *in vivo* antifungal responses.

**Giuseppe Fleres**, **Shaoji Cheng**, **Hassan Badrane**, **Chris L Dupont**, **Binghua Hao**, **Anthony Newbrough**, **Cornelius J Clancy**, **Minh Hong Thi Nguyen**

1University of Pittsburgh, PA, USA. 2Department of Human Biology and Genomic Medicine, J. Craig Venter Institute, La Jolla, CA 92037, USA. 3University of Pittsburgh Medical Center (UPMC), USA

**Abstract**

Background: Within-patient Ca genotypic diversity has been demonstrated during oral cavity colonization. We evaluated within-patient diversity of Ca from BSIs, which are acute infections of a sterile site.

Methods: We performed Illumina WGS of 10 CA strains recovered from blood cultures (BCs) of each of 4 patients. We assessed antifungal responses and filamentation *in vitro*, and echinocandin responses during Galleria infections.

Results: Strains belonged to 4 clades, and clustered by patient (Pt). Within-patient strains were distinct, differing by ≥4,460 SNPs. Genes associated with cell wall, adhesion, white-opaque switching, filamentation and nutrient transport (e.g., *hyr1*, *als1*, *hst1*, *fgr2*) had mutations that distinguished between strains in all patients (i.e., found in ≥1 and <9 strains/Pt). Segmental loss of heterozygosity of Chr2, Chr4 or Chr6 was observed in 3/10 strains from Pt1. Aneuploidy was observed in 4/10 (Chr7) and 1/10 (Chr5) strains from Pt2 and Pt3, respectively. Aneuploid strains from Pt2 had SNP/indels in GPI-anchored Hyp1p, mismatch-repair and core stress response genes, which were not seen in other Pt2 strains. Aneuploid Pt2 and Pt3 strains did not form hyphae on Spider medium, and were more susceptible to echinocandins and other cell wall-perturbing agents *in vitro* (Congo red, SDS). They were more susceptible and resistant to azoles, respectively. *Galleria* infected with an aneuploid Pt2 strain had significantly greater responses to micafungin than did a non-aneuploid Pt2 strain, as measured by survival (p=0.007, log-rank/Kaplan-Meier), and *Candida* burdens.

Conclusion: CA from BSIs demonstrated within-patient diversity in genotypes and phenotypes important for pathogenesis and antifungal responses.
Towards an *in vitro* gut model to study the interplay between *Candida* species and bacteria in eubiosis and dysbiosis.

**Benoît Marsaux**¹,², Frédéric Moens², Massimo Marzorati¹,², Tom Van de Wiele¹,²

¹Center for Microbial Ecology and Technology (CMET), Department of Biotechnology, Faculty of Bioscience Engineering, University of Ghent, Belgium. ²ProDigest B.V., Belgium

**Abstract**

*Candida albicans* is a commensal yeast commonly found in the gastrointestinal tract of healthy humans that can turn pathogenic under predisposing conditions. The commensal-to-pathogen transition is correlated with *C. albicans* overgrowth. This can occur following dysbiotic events, such as antibiotic treatments.

Although we suspect that the environmental pressure of a healthy microbiome limits fungal expansion, little is known about which microbiome members contribute the most to this reduction.

Here we use the Simulator of the Human Intestinal Microbial Ecosystem® (SHIME®). Using faecal samples as inoculum, it allows the culturing of the human microbiome over several weeks and under conditions representative for different intestinal regions. We monitored the concentration of four *Candida* species (*C. albicans, C. tropicalis, C. parapsilosis, C. glabrata*) on distinct individuals by qPCR, in eubiosis and dysbiosis. We further investigated the impact of three antibiotic treatments on *C. albicans* level, and monitored bacterial diversity change by 16S rRNA gene sequencing.

We observed *Candida* species-specific engraftment under eubiosis, whereas growth of all species was achieved upon antibiotic treatment, reaching similar fungal load as observed *in vivo*. In addition, we found that only some antibiotics lead to elevated *C. albicans* level, and their impact is donor dependent.

Our next step will be to isolate bacterial signatures that are correlated with higher risk of *Candida* overgrowth, either at taxonomic or predictive functional level. This would ultimately lead to the identification of live biotherapeutic products.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme (grant No 812969).
Post-transcriptional regulation of gene expression during morphological transition in *Candida albicans*

Anushka Nayak, Sohail Khoshnevis

Emory University School of Medicine, USA

Abstract

Background: *Candida albicans*, an opportunistic fungal human pathogen, is a major threat to the healthcare system due to both infections in immunocompromised individuals and the emergence of antifungal resistance. *C. albicans* undergoes a morphological transition from yeast to hyphae and this transition is important for its virulence. Numerous studies of cell-type switching have defined transcriptional programs critical to control this transition, but little is known about the role of post-transcriptional processes in regulating this critical morphological transition. Here, we investigate the hypothesis that post-transcriptional RNA modifications play an important role in controlling the morphological transition of *C. albicans*.

Results: Using RNA isolated from different timepoints along the hyphae formation, we show that RNA methylation levels change during morphological transition in *C. albicans*. We determine the methyltransferase enzyme responsible for these methylations and demonstrate that the expression of this methyltransferase enzyme depends on the morphological state of *C. albicans*. Deletion of this enzyme alters the transcriptional landscape of *C. albicans* and results in reduced hyphae development, biofilm formation and adhesion to the epithelial cells. Small molecule inhibition of this enzyme reduces the RNA methylation during hyphae formation and impairs many features of *C. albicans* associated with its pathogenicity without affecting the human epithelial cells, thereby opening new windows for targeting *Candida* infections.

Conclusions: Our data suggest a role for post-transcriptional RNA methylation in controlling the yeast-to-hyphae transition in *C. albicans*. We are working towards exploiting this mechanism as a novel therapeutic avenue against *Candida* infection.
Characterizing the Oral Mycobiome of Domestic Dogs

Elisa Rouse-Salcido¹, Brooke Esquivel¹, Brandon Holder¹, Butch KuKanich², Kate KuKanich², Allison Schweiker¹, Dariyan Springfield², Theodore C. White¹

¹University of Missouri-Kansas City, USA. ²Kansas State University, USA

Abstract

Understanding the oral and gut mycobiome is a relatively new field of study in humans and animals. The purpose of this study is to characterize the oral mycobiome of domestic dogs to identify commensal and potentially pathogenic fungi present.

253 buccal swabs were obtained in collaboration with Kansas State University’s College of Veterinary Medicine and a local animal shelter. Swabs were struck onto a chromogenic fungal growth medium that distinguishes between fungal species based on colony color and morphology. After isolating and harvesting colonies, DNA was extracted from each species. PCR was used to amplify a fungus-specific variable region of the genome (ITS-1), which was then sequenced. NCBI BLAST database was used to identify each species present. Multiple human pathogens in the Candida genus were identified, including C. albicans, C. glabrata, C. parapsilosis, and C. auris as well as other pathogenic fungi, predominantly Malassezia species. Isolation of C. auris from a dog mouth is particularly interesting and concerning.

After the isolates were collected, cultured, and speciated, we began antifungal drug susceptibility testing against common drugs such as fluconazole, ketoconazole, and terbinafine.

Exploring the oral mycobiome of dogs as well as the corresponding drug susceptibility of isolates will allow researchers to assess the appropriateness of antifungal use as it relates to drug resistance in animals and humans. These findings will improve our understanding of the microorganisms within our pets, and thus the organisms that humans are commonly exposed to through our canine companions.
Fighting back against fluconazole resistant *Candida albicans* with iron chelator, deferasirox

Rishabh Sharma, Anubhav Nahar, Sumant Puri

Temple University, Oral Health Sciences, USA

Abstract

Objective: Widespread use of fluconazole has resulted in the emergence of fluconazole-resistant *Candida* spp. infections that are hard to treat. This study aims to investigate the role of iron chelator deferasirox (DFX) in the treatment of fluconazole-resistant *C. albicans*.

Methods: A fluconazole-resistant (FLC-R) strain of *C. albicans* was generated by passaging in presence of increasing fluconazole concentrations. Effect of DFX on FLC-R strain was assessed by colony forming units (CFU) on YNB-agar plates containing 128 µg/ml fluconazole with or without 4.66 µg/ml DFX. Gene expression in wild-type (WT) and FLC-R strains grown in low iron medium (LIM) and high iron medium (RIM) was measured using qPCR. Intracellular iron was evaluated using an iron kit (MAK025). Cell wall studies were performed by staining with Concanavalin A (mannan), β-glucan antibody (β-1,3-glucan), and Calcofluor white stain (chitin).

Results: FLC-R strain was unable to survive in presence of the sub-lethal dose of the DFX and showed enhanced accumulation of intracellular iron in LIM (43%) and RIM (46%), in comparison to the WT. Ergosterol biosynthesis pathway gene *ERG11* was up-regulated in FLC-R strain. Expression of iron-acquisition genes (*FRP1*, *RBT5* and *SIT1*) was up-regulated while iron-utilization genes (*BIO2* and *ACO11*) was down-regulated in FLC-R strain. β-glucan exposure was significantly augmented in FLC-R strain, compared to WT.

Conclusion: Fluconazole-resistant strain of *C. albicans* has a greater demand for cellular iron. Treatment with DFX can provide an alternative antifungal treatment-strategy for such strains.
Arginine induces *Candida albicans* filamentation through a mitochondrial respiration independent mechanism.

Zhongle Liu, Miaoxi Liu, Saif Hossain, Nicole Robbins, Leah Cowen

University of Toronto, Canada

Abstract

Amino acids such as arginine and proline can induce yeast-to-hyphae transition, a key virulence trait of the human fungal pathogen *Candida albicans*. Under aerobic conditions, robust hyphal induction requires high levels of amino acids. These amino acids are catabolized through mitochondrial respiration to increase the cellular ATP level, which in turn activates the protein kinase A pathway to promote filamentous growth. Here, we report that low micromolar levels of arginine induces *C. albicans* filamentation under anaerobic conditions. Lack of molecular oxygen, the essential electron acceptor of the electron transport chain, blocks respiration and prevents arginine degradation to produce ATP. Therefore, our finding suggests the presence of a novel filamentation inducing mechanism for arginine that is independent of mitochondrial respiration. Further exploration revealed that the arginase (Car1), the enzyme degrading arginine into ornithine and urea, is required for arginine-induced anaerobic filamentation, while ornithine aminotransferase (Car2) and urea amidolyase (Dur1,2), the enzymes further breaking down the arginase reaction products, are dispensable for filamentation. Additionally, we performed a functional-genomic screen which identified Arg81 as a key transcription factor mediating arginine induced anaerobic hyphal growth. This work expands our knowledge of how *C. albicans* morphology is linked to the metabolic state, which is contingent on nutrient and oxygen availability.
Cross-Kingdom interactions between Candida albicans and Enterococcus faecalis in the Gut Microbiome

Haley Gause, Alexander Johnson

University of California, San Francisco, USA

Abstract

Candida albicans and Enterococcus faecalis are both common members of the human gut microbiome. Previous studies have suggested that this yeast and bacteria species interact as commensal members in the gut, however the mechanisms behind these interactions have yet to be elucidated. By measuring gene expression of these species in co-culture, we can start to tease apart the genetic underpinnings of this interaction. Using Dual RNA-seq, we characterized the transcriptional profiles of both C. albicans and E. faecalis during growth together and separately in two conditions – 1) gut-like in-vitro culture and 2) Germ-free (GF) mouse gut. The transcriptional response of C. albicans to E. faecalis is very similar across in vitro and GF gut conditions, with over 300 genes up-regulated > 4-fold in either condition. Up-regulated genes include a group of seven transcription factors which are induced exclusively in the presence of E. faecalis. Many of these transcription factors are currently uncharacterized, indicating a possible function in mediating interkingdom interactions with bacteria in biologically relevant environments, such as the gut. Future work is ongoing to determine the transcriptional networks controlled by these upregulated transcription factors and the role of those networks in the interactions between Candida albicans and Enterococcus faecalis.
Multiple phosphorylation sites in Sfl1 integrate specific environmental signals to determine C. albicans cell fate

Misty Peterson, Haoping Liu
University of California, Irvine, USA

Abstract

Morphological flexibility facilitates the ability of the pathogen C. albicans to adapt to environmental stimuli. This is driven by a network of transcription factors that regulate each other and drive yeast or hypha specific gene expression. Hypha initiation requires a cAMP-dependent Protein kinase A (PKA) contingent down regulation of NRG1 (repressor of hyphal development) transcription, as well as the temporarydownregulation of Nrg1 protein for establishment of a hyphal transcription state. Mitogen-activated protein kinases (MAPK) are activated in response to various stresses, including cell wall and osmotic stresses. We have previously linked Sfl1 repression of hyphal initiation to stress responsive MAPKs.

Here we report that multiple phosphorylation sites in Sfl1 integrate cAMP/PKA and MAPK signals to regulate hyphae initiation. We observe that phosphomimetic substitution in the conserved PKA site of the Sfl1 HSD (heat shock domain) allowed hyphal initiation whereas the non-phosphorylatable mutation at the PKA site of SFL1 showed a lower level of hyphal initiation than the SFL1 control. This supports that PKA phosphorylation of Sfl1 dissociates the repressor from DNA. A phosphomimetic sfl1 mutant with substitutions in conserved MAPK sites of the Sfl1 HSD showed less Sfl1 protein and bypass of hyphal inhibition by Sfl1. A matching non-phosphorylatable sfl1 mutant showed stronger inhibition of hyphal initiation relative to the wild-type SFL1 control. Therefore, phosphorylation in the HSD of Sfl1 by either the cAMP/PKA or MAPK pathways can remove Sfl1 inhibition and promote hyphal initiation. Our ongoing experiments aim to determine which MAPK and associated upstream signals regulate Sfl1.
Als adhesin proteins of *Candida albicans* hyphae trigger Neutrophil Extracellular Traps (NETs)

Rachel Garleb¹², Tingting Zhou¹, Michaela Marshall³, Mengli Yang¹⁴, Scott Filler⁵⁶, Eric Pearlman³², Haoping Liu¹²

¹Department of Biological Chemistry, School of Medicine, University of California, Irvine CA92617, USA. ²Institute for Immunology, University of California, Irvine CA92617, USA. ³Department of Physiology and Biophysics, University of California, Irvine, California, United States of America, USA. ⁴School of Pharmacy & Pharmaceutical Sciences, University of California, Irvine, California, United States of America., USA. ⁵Division of Infectious Diseases, Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, California, United States of America., USA. ⁶David Geffen School of Medicine at UCLA, Los Angeles, California, United States of America., USA

Abstract

Neutrophils are critical for the clearance of *Candida albicans* infection, and patients with low neutrophil counts (neutropenia) show higher susceptibility to invasive candidemia. Neutrophils effectively clear *C. albicans* yeast by phagocytosis through recognition of cell wall β-glucan. However, neutrophils are unable to phagocytize the larger hyphal form and undergo NETosis (neutrophil extracellular trap), and this mechanism is not well understood.

To determine if hyphal expressed proteins play a role in NETosis, we screened *C. albicans* mutants for NET induction in C57BL/6 neutrophils through SYTOX extracellular DNA quantification. We discovered neutrophils stimulated by the als3 mutant is impaired in NET release, which is further impaired by the als1als3 double mutant. To examine Als-mediated NETosis and hyphal killing, we generated a triple Als mutation (als5als3S¹⁷⁰Yals1S¹⁷⁰Y) by CRISPR/Cas9 point mutation in the peptide-binding cavity of Als3 and Als1 that disrupts Als adhesive function. We found neutrophils stimulated by als5als3S¹⁷⁰Yals1S¹⁷⁰Y was significantly defective in histone H3 citrullination and unable to induce extracellular DNA release, which are hallmarks of classical NETosis. Since NETosis is known to play a role in neutrophil killing of hyphae, we assessed in-vitro survival of *C. albicans* hyphae after neutrophil co-incubation with *C. albicans* hyphae. We discovered Als proteins are required for neutrophil killing of *C. albicans* hyphae, as the als5als3S¹⁷⁰Yals1S¹⁷⁰Y triple mutation strain has substantially increased survival after co-incubation compared to the WT strain. Current ongoing experiments are to determine which inflammatory cell death pathways are responsible Als-mediated NETosis and *C. albicans* hyphae killing.
Trends of antifungal resistance and tolerance of *Candida* bloodstream isolates in Taiwan

**PAO-YU CHEN**<sup>1,2</sup>, **YEE-CHUN CHEN**<sup>1,3,4</sup>

<sup>1</sup>Department of Internal Medicine, National Taiwan University Hospital, Taiwan.  <sup>2</sup>Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taiwan.  <sup>3</sup>National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Taiwan.  <sup>4</sup>Department of Medicine, National Taiwan University College of Medicine, Taiwan.

**Abstract**

**Background**

Antifungal resistance and tolerance are two different phenotypes by in vitro susceptibility testing, and might be predictive of therapeutic outcomes. The surveillance of antifungal tolerance in *Candida* remained few. This study depicted secular trends of antifungal resistance and tolerance of 1276 non-duplicated *Candida* bloodstream isolates collected at a single center in every other year between 2011 and 2021.

**Materials**

Antifungal susceptibility testing was performed by the Sensititre YeastOne SYO-10 panel, and the results were interpreted according to CLSI breakpoints (BPs) or epidemiological cutoff values (ECVs). Azole trailing was defined by MIC<sub>48</sub>/MIC<sub>24</sub> ≥ 8 and switch of BPs category from susceptible/susceptible-dose dependent to resistant or ECVs category from wild type (WT) to non-WT. Paradoxical growth (PG) was defined by regrowth of *Candida* at high concentrations of echinocandin after 48-hour incubation period.

**Results**

Overall, the proportions of *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. parapsilosis* was 43.8%, 24.8%, 18.6%, and 12.8%, respectively. Both fluconazole/voriconazole resistance and trailing were commonly observed in *C. tropicalis* (18.3%/17.7% and 37.2%/44.8%), as well as in *C. glabrata* (21.1%/51.9% and 22.8%/24.5%). The trends of resistance and trailing significantly increased in both species. Despite echinocandin resistance remained low (<1%) among all *Candida* species, 62.1% and 59.0% of *C. tropicalis* showed PG to anidulafungin/micafungin compared to <1% in the rest *Candida* spp. Also, the trend of PG in *C. tropicalis* significantly increased.

**Conclusion**

*C. tropicalis* demonstrated not only resistance to azole but also tolerance to azole and echinocandin, while *C. glabrata* showed azole resistance and tolerance only.
Candida auris cell wall proteome characterisation and effectiveness against hematogenously disseminated candidiasis in murine model

Vartika Srivastava¹, Aijaz Ahmad¹,²

¹Department of Clinical Microbiology and Infectious Diseases, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. ²Division of Infection Control, Charlotte Maxeke Johannesburg Academic Hospital, National Health Laboratory Service, Johannesburg, South Africa

Abstract

Candida auris is emerging as a pan-resistant pathogenic yeast among immunocompromised patients worldwide. Early diagnostics and vaccines are the best alternatives to handle this drug resistant pathogen. Cell wall proteins are responsible for eliciting immune response in host and therefore can be promising candidates for the development of therapeutic biomarkers and vaccines against C. auris. In present study we performed antifungal susceptibility profiling against standard antifungal drugs using CLSI guidelines. Also, all the clinical strains (n=10) were screened for the presence of various virulence factors. Based on the results the most resistant strain of C. auris was selected for extraction of cell wall associated proteins. The cell wall proteome was characterized by using LC-MS followed by bioinformatic and functional analyses. Furthermore, the protective effect of the cell wall associated protein against C. auris infection was evaluated in BALB/c female mice. Based on the antifungal susceptibility profiling and expression of virulence attributes C. auris MRL6057 was selected for cell wall associated protein extraction and in vivo studies. A total of 60 proteins were predicted to be immunodominant. In vivo results demonstrated lowered fungal burden in target organs and increased survival in vaccinated mice as compared to the infection control group revealing the immunogenic property of cell wall associated proteins. Therefore, this study validates the mass-spectrometry approach to identify antigenic proteins of C. auris and the potential of these proteins to serve as biomarkers for development of diagnostic assay and/or vaccines against infections caused by C. auris.
A tractable nematode model for the emerging fungal pathogen, *Candida auris*

Melissa Martinez1,2, Melissa Cruz2, Pedro Miramon-Martinez2, Danielle Garsin2, Michael Lorenz2

1University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences, USA. 2University of Texas Health Science Center at Houston, McGovern Medical School, Department of Microbiology and Molecular Genetics, USA

Abstract

*Candida auris* is an emerging multidrug-resistant fungal pathogen. The genetic factors that contribute to the virulence, drug resistance, and stress-tolerant nature of *C. auris* are almost completely unknown. Current murine models of *Candida auris* infections are not ideal. Mice must be immunosuppressed, and the long time-course of this model leaves animals susceptible to other infections that complicate analysis. Additional relevant animal models, especially ones amenable to high throughput analysis are needed. The nematode *Caenorhabditis elegans* is a tractable model organism that has been validated as a valuable tool for studying fungal pathogenesis. Its fast generation time, well-established genetics, low cost, and the ease of delivering the pathogen makes this an ideal model for high-throughput analysis of virulence. We have developed a *C. elegans* assays for *C. auris* virulence, in which survival of infected nematodes is used to assess the pathogenicity of the fungus. In this study, we have shown that strains from at least three clades rapidly kill worms. Moreover, we show that this model can distinguish differences in virulence between strains. We are now using the high-throughput version of this model that utilizes live-dead staining to screen an insertionional mutation library we have created using *Agrobacterium tumefaciens*-mediated transformation as well as a clinical isolate collection provided by the Centers for Control and Disease Prevention for strains exhibiting attenuated or unique virulence patterns.
An Adjuvant-Based Approach Enables the Use of Dominant HYG and KAN Selectable Markers in Candida albicans

Corey Frazer, Sang On Park, Richard Bennett

Brown University, USA

Abstract

The genetic tractability of Candida albicans has enabled significant progress in our understanding of its biology and pathogenesis. However, C. albicans is inherently resistant to antifungals commonly used for selection of genetic modifications in other fungi, allowing high background growth of untransformed cells. Only a single dominant selectable marker (nourseothricin/ClonNAT) has seen widespread use by the field, severely limiting molecular genetics tool development compared to model fungi like Saccharomyces cerevisiae. Here we show that quinine and molybdate (inhibitors of amino acid and sulfate transport, respectively), act synergistically with the aminoglycoside antibiotics G418 and Hygromycin to inhibit C. albicans growth. This enables the use of CaHygB and CaKan selectable markers for genetic manipulation by suppressing the growth of non-transformed cells. We show that hygromycin and G418 resistance are orthogonal to nourseothricin resistance, greatly expanding the number of dominant markers usable in the study of this organism. Furthermore, we show that neither quinine nor molybdate are significantly mutagenic.
Conserved role of ferric reductases in heme utilization

weissman ziva¹, Daniel Kornitzer²

¹Technion Faculty of medicine, Israel. ²Technion, Israel

Abstract

To overcome iron limitation in the host environment, many Candida spp. are able to utilize iron from hemoglobin, the largest iron pool in the human body. To achieve this, they express an extracellular cascade of soluble and cell surface - unchored CFEM-domain hemophores-Pga7, Rbt5 and Csa2, that extract heme from hemoglobin and transfer it to the plasma membrane. Frp1 and Frp2, membrane proteins related to ferric reductases, function with the CFEM hemophores in heme internalization. Ferric reductases are ubiquitous in eukaryotes, but were not previously associated with heme transport. Here we addressed the question wether ferric reductases outside the Candida clade could be involved in heme transport as well. We observed that the CFEM hemophore family of proteins was initialy identifird in a screen for C.albicans genes that could confer hemoglobin utilization to S.cerevisiae. Since no C.albicans ferric reductase-like protein were required for S.cerevisiae hemoglobin utilization under these conditions, we surmised that a S.cerevisiae ferric reductase might colaborate in heme utilization with the ectopically expressed Candida CFEM hemophore. Targeted screening of the nine suspected S.cerevisiae ferric reductase genes identifide one, FRE5, that is required for hemoglobin utilization in the presence of the C.albicans hemophore. Furthermore, FRE5 is also required for efficient utilization of free hemin by S.serevisiae. We concluded that the role of ferric reductase -like proteins in extracellular heme uptake and / or intracellular heme transport is widespread and not restricted to fungal parasites of animals.
**C207**

**Epl1, a subunit of the NuA4 acetyltransferase complex, negatively regulates yeast-to-hyphal transition of *Candida albicans***

Guolei Zhao¹, Teresa O’Meara², Judith Berman³, Laura Rusche¹

¹University at Buffalo (SUNY), USA. ²University of Michigan, USA. ³Tel Aviv University, Israel

**Abstract**

The ability of *Candida albicans* to adopt both yeast and filamentous growth forms is critical for its pathogenesis. Previously, we found that the sirtuin deacetylase Sir2 and its deacetylase activity promote the induction of hyphal growth of *C. albicans*. To understand how Sir2 contributes to hyphal growth, we focused on Epl1, one of the nonhistone targets of Sir2 recently identified in an acetylome study of *C. albicans*. Epl1 is an essential component of the NuA4 acetyltransferase complex, which associates with the promoters of hyphal-specific genes and regulates their expression. The acetylome study showed that lysines 10, 14, 16, and 32 of Epl1 are deacetylated by Sir2. Lysine 32 is highly conserved among all fungal Epl1 orthologs examined, whereas lysines 10, 14, and 16 are conserved among species in the CTG-Ser1 clade. Deleting the segment of *EPL1* from K10 to K16 resulted in hyper-filamentous growth, as did shutting off expression of *EPL1*. Consistent with its negative role in hyphal growth, Epl1 protein level was higher in yeast compared to hyphal cells, particularly when *SIR2* was deleted. In conclusion, the results suggest that Epl1 negatively regulates the yeast-to-hyphae transition.
Population genetics and microevolution of clinical *C. glabrata* isolates reveals recombinant sequence types and hyper-variation within mitochondrial genomes, virulence genes, and drug targets

Nicolas Helmstetter¹, Aleksandra Chybowska², Christopher Delaney³, Alessandra Da Silva Dantas¹, Hugh Gifford¹, Theresa Wacker¹, Carol Munro², Adilia Warris¹, Brian Jones⁴, Christina Cuomo⁵, Duncan Wilson¹, Gordon Ramage³, Rhys Farrer¹

¹Medical Research Council, Centre for Medical Mycology, University of Exeter, United Kingdom. ²Institute of Medical Sciences, University of Aberdeen, United Kingdom. ³School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, United Kingdom. ⁴Institute of Infection, Immunity & Inflammation, University of Glasgow, United Kingdom. ⁵Broad Institute of MIT and Harvard, USA

Abstract

Genome analysis of 68 isolates of *Candida glabrata* from 8 hospitals across Scotland, together with 83 global isolates, revealed insights into the population genetics and evolution of *C. glabrata*. Clinical isolates of *C. glabrata* from across Scotland are highly genetically diverse, including at least 19 separate sequence types that have been recovered previously in globally diverse locations, and 1 newly discovered sequence type. Several sequence types had evidence for ancestral recombination, suggesting transmission between distinct geographical regions has coincided with genetic exchange arising in new clades. Three isolates were missing MATa1, potentially representing a second mating type. Signatures of positive selection were identified in every sequence type including enrichment for epithelial adhesins thought to facilitate fungal adhesin to human epithelial cells. In patent microevolution was identified from 7 sets of recurrent cases of candidiasis, revealing an enrichment for non-synonymous and frameshift indels in cell surface proteins. Microevolution within patients also affected epithelial adhesins genes, and several genes involved in drug resistance including the ergosterol synthesis gene ERG4 and the echinocandin target FKS1/2, the latter coinciding with a marked drop in fluconazole minimum inhibitory concentration. In addition to nuclear genome diversity, the *C. glabrata* mitochondrial genome was particularly diverse, with reduced conserved sequence and conserved protein encoding genes in all non-reference ST15 isolates. Together, this study highlights the genetic diversity within the *C. glabrata* population that may impact virulence and drug resistance, and 2 major mechanisms generating this diversity: microevolution and genetic exchange/recombination.
C210

Effects of Inflammation on Host-Fungal Interactions at the Base of Animal Evolution

Sarina Qin, Juris Grasis, Clarissa Nobile
University of California Merced, USA

Abstract

*Candida albicans* is one of the most common fungal pathogens of humans, capable causing severe infections particularly in immunocompromised individuals. Although much has been learned about the roles of the adaptive immune system during *C. albicans* infection using mouse models of systemic infection, less is known about the specific roles of the innate immune system, particularly in terms of host inflammatory states. In this project, we use *C. albicans* to investigate infections of the innate immune model organism *Hydra*, a primitive animal natively devoid of an adaptive immune system. By evaluating different stages of infection and recovery, we are investigating candidate innate immune regulatory genes and pathways specifically associated with *C. albicans* infection. To further expand the infection model, inflammation-like states are being induced to simulate an “immunocompromised-like” state in the primitive *Hydra* host. Overall, this project will give insight into the roles of inflammation in an innate immune model system during infection and recovery. It will also shed new light on the key host players involved to clear infections during inflamed states as well as identify *C. albicans* target genes involved in virulence mechanisms specific to the innate immune system.
Intestinal Colonization with Candida auris and Mucosal Immune Response in Mice Treated with Cefoperazone Oral Antibiotic

Diprasom Das, Harm HogenEsch, Shankar Thangamani
Purdue University, USA

Abstract

Candida auris, an emerging multi-drug resistant fungal pathogen, causes invasive infections in humans. The factors regulating the colonization of C. auris in host niches are not well understood.. Our results indicate that mice treated with cefoperazone alone had a significant increase in C. auris intestinal colonization compared to untreated control groups. A significant increase in the dissemination of C. auris from the intestine to internal organs was observed in antibiotic-treated immunosuppressed mice. Intestinal colonization of C. auris alters the microbiome composition of antibiotic-treated mice. Relative abundance of firmicutes members mainly Clostridiales and Paenibacillus were considerably increased in the cefoperazone-treated mice infected with C. auris compared to cefoperazone-treated uninfected mice. Next, we examined the mucosal immune response of C. auris infected mice and compared the results with Candida albicans infection. The number of CD11b+ CX3CR1+ macrophages was significantly decreased in the intestine of C. auris infected mice when compared to C. albicans infection. On the other hand, both C. auris and C. albicans infected mice had a comparable increase of the number of Th17 and Th22 cells in the intestine. Significant increase in the Candida-specific IgA was observed in the serum of C. auris but not in the C. albicans infected mice. Taken together, treatment with broad-spectrum antibiotic increased the colonization and dissemination of C. auris from the intestine. Furthermore, findings from this study for the first time revealed the microbiome composition, innate and adaptive cellular immune response to intestinal infection with C. auris.
Differential regulation of the antifungal response by Tra1 in Candida albicans

Gabriela Nunes Marsiglio Librais¹, Rebecca S. Shapiro², Patrick Lajoie³

¹University of Western Ontario, Canada. ²University of Guelph, Canada

Abstract

Candida albicans infection is the leading cause of mortality from fungal infections, and the emergence of resistant strains decreases the effectiveness of first-line therapy, such as echinocandins. Tra1 is a conserved and essential component of the SAGA and NuA4 transcriptional co-activator complexes that regulate gene expression during various stress conditions and have been shown to regulate C. albicans antifungal response and pathogenicity. Here, we used C. albicans strains carrying a loss-of-function allele of TRA1 (tra1Q3). We previously demonstrated that mutations in TRA1 reduce pathogenicity and increase caspofungin sensitivity in C. albicans (Razzaq et al., 2021, Genetics). Unexpectedly, we found that a lack of Tra1 function increases resistance to azoles in C. albicans. This contrasts with the increasedazole sensitivity observed for a similar mutant in S. cerevisiae. This highlights differential regulation of the antifungal response by Tra1 across yeast species. In C. albicans, tra1Q3 is associated with increased expression of genes linked to azole resistance, such as ERG11 and CDR1, a membrane transporter associated with the efflux of chemicals, including azoles. Consequently, we found that tra1Q3 cells display increased efflux of the Cdr1 substrate rhodamine 6G. Furthermore, analysis of fluorescently-labelled azole uptake reveals that tra1Q3 cells show reduced accumulation of intracellular fluconazole. C. albicans Tra1 therefore differentially modulates the response to various antifungal drugs. Supported by NSERC and CIHR.
The effect of isochromosome 5L on Candida albicans gene expression and response to fluconazole

Petra Vande Zande, Hung-Ji Tsai, Anjali Nelliat, Rob Todd, Anna Selmecki

University of Minnesota, USA

Abstract

Candida albicans can adapt to azoles by undergoing large-scale genomic amplification events that simultaneously increase the copy number of many genes. The formation of an isochromosome of the left arm of chromosome 5 (i(5L)) is a recurrent amplification identified in azole-resistant clinical isolates and in vitro evolved isolates. The i(5L) causes azole resistance via amplification of the gene encoding the azole drug target, ERG11, and a transcriptional regulator of drug efflux pumps, TAC1. However, how the isochromosome changes global gene expression in response to drug is not known. To determine the genome-wide impact of i(5L) on gene expression we collected RNA-sequencing data for a clinical isolate with and without the isochromosome, both in the presence and absence of fluconazole. We find that the isolate bearing i(5L) does not show a gene expression pattern related to hypo-osmotic stress as seen in Saccharomyces cerevisiae with acute aneuploidization. As expected, expression levels of ERG11 and TAC1, located on 5L, are upregulated in proportion to their increase in copy number. When exposed to fluconazole, the entire ergosterol biosynthesis pathway is upregulated more highly in the progenitor strain than in the i(5L)-bearing strain. In contrast, TAC1 and its targets CDR1 and CDR2 do not change in response to fluconazole but are already upregulated in the i(5L)-bearing strain relative to the progenitor. These data help explain the relatively low fitness cost of the isochromosome in rich media and the fitness benefit that it confers in low concentrations of drug.
Outcomes of Subjects with Refractory Candida Infections Treated with Oral Ibrexafungerp requiring Extended Therapy from an Interim Analysis of FURI

Nkechi Azie, Oliver A Cornely, Riina Rautemma-Richardson, Thomas J Walsh, David A Angulo

1SCYNEXIS, Inc., USA. 2University of Cologne, Germany. 3University of Manchester, United Kingdom. 4Center for Innovative Therapeutics and Diagnostics, Richmond, VA, USA

Abstract

Introduction: Ibrexafungerp (IBX) is an investigational broad-spectrum glucan synthase inhibitor triterpenoid antifungal with activity against Candida spp., Aspergillus spp, including azole- and echinocandin-resistant strains. A global Phase 3 open-label, single-arm study of oral ibrexafungerp (FURI) (Clinicaltrials.gov NCT03059992) has been completed. We present an analysis of subjects from this study with refractory fungal diseases or intolerance to previous therapy with Candida infections that received greater than 90 days of IBX.

Methods: An independent Data Review Committee assessed treatment responses for 113 subjects. An interim analysis of 22 subjects with invasive (IC) or severe mucocutaneous candidiasis (MC) that required IBX therapy for >90 days was performed. Patients were eligible for enrollment if they had proven or probable IC or MC and documented evidence of failure of, or intolerance to a currently approved standard-of-care antifungal treatment.

Results: Among 22 subjects, 12 were enrolled with MC (9 oropharyngeal, 2 esophageal, 1 skin/nail) and 10 with IC (6 osteo-articular, 4 visceral). The mean duration and range of IBX therapy was 115 days (90 days – 180 days). Most patients had candidiasis refractory to previous therapy (16) and 6 were intolerant. The most common Candida spp. was Candida albicans in 50%. Outcomes for this patient group were Complete or Partial Response 14/22 (64%), Stable Response 6/22 (27%) and Progression of Disease 2/22 (9%). There were no drug-related serious adverse events or discontinuations due to adverse events.

Conclusion: IBX demonstrated favorable outcomes and was well-tolerated in refractory IC and MC in subjects requiring extended IBX therapy (>90 days).
Characterizing Candida albicans hyphal regulation in the context of host macrophages

Hannah Wilson1,2, Michael Lorenz1

1University of Texas Health Science Center at Houston, USA. 2MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences, USA

Abstract

The interaction between Candida albicans and macrophages is dynamic and complex. Once phagocytosed, C. albicans engages alternative metabolic pathways and rapidly adapts to the phagosomal environment. The fungus neutralizes the acidic phagosome and induces morphogenesis, generating hyphae that physically rupture and kill the macrophage. The signals that drive hyphal growth in this context have not yet been identified, although several models have been implicated. In this study, we test CO2, intracellular pH, and extracellular pH as potential inducers of morphogenesis within macrophages. Using live-imaging microscopy, we assessed the hyphal phenotypes of the C. albicans CO2-sensing mutants nce103Δ/Δ (lacking the enzyme carbonic anhydrase) and a cyr1 point mutant (possessing a bicarbonate-insensitive adenylyl cyclase). These mutants generated hyphae after phagocytosis, suggesting that CO2-sensing is dispensable for this process. In order to ask if intracellular pH could be a regulator of morphogenesis, we generated a C. albicans strain expressing the pH probe pHluorin2 cytosolically. Ratiometric pH measurements were acquired over the course of hyphal morphogenesis both in vitro and inside of macrophages. The fungal cytosol remained near neutral in all cases, suggesting that intracellular pH changes do not regulate hyphal morphogenesis. Concurrently, in order to ask if extracellular pH sensing is important for this process, we tested a rim101Δ/Δ mutant in co-culture and saw no hyphal defect. Thus, neither CO2 sensing nor pH fluctuations are required for the induction of morphogenesis in this context. Work is ongoing to reveal the signal(s) driving this process.
The ADR1 transcription factor directs regulation of the ergosterol pathway and azole resistance in *Candida albicans*

Gaëlle Kouyoumdjian¹, Manjari Shrivastava²¹³, Eftyhios Kirbizakis¹, Daniel Ruiz¹, Manon Henry²³, Antony Vincent⁴, Adnane Sellam², Malcolm Whiteway¹

¹Concordia University, Canada. ²Montreal Heart Institute, Canada. ³Department of microbiology, infectiology and immunology, faculty of medicine, Université de Montréal, Canada. ⁴Department of Animal Sciences Université Laval, Canada

Abstract

Transcription factors play key roles in cellular regulation and are critical in the control of drug resistance in the fungal pathogen *Candida albicans*. We found that activation of the transcription factor C4_02500C_A (ADR1) conferred significant resistance against fluconazole. However, in *Saccharomyces cerevisiae* ADR1 is a carbon-source-responsive zinc-finger transcription factor required for transcription of the glucose-repressed gene ADH1 and of genes required for ethanol, glycerol, and fatty acid utilization. Motif scanning of promoter elements suggests that ADR1 may be rewired in the fungi and involved in the ergosterol synthesis pathway in *C. albicans*. Because previous studies have identified the zinc-cluster transcription factor UPC2 as a regulator of the ergosterol pathway in both fungi, we examined the relationship of ADR1 and UPC2 in sterol biosynthesis in *C. albicans*. Phenotypic profiles of either ADR1 and UPC2 modulation showed differential growth in the presence of fluconazole; either ADR1 or UPC2 homozygous deletion results in sensitivity to the drug while their activation generates a fluconazole resistant strain. The rewiring from ergosterol synthesis to fatty acid metabolism involved all members of the ADR1 regulon except the alcohol dehydrogenase ADH1, which remains under ADR1 control in both circuits and may have been driven by the lifestyle of *S. cerevisiae*, which requires the ability to both tolerate and process high concentrations of ethanol.