Candida and Candidiasis

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#Candida2025

POSTER ABSTRACT BOOK

MICROBIOLOGY SOCIETY

Elevator talk

BLOCK A

Members of the *TLO* (telomere-associated) expanded gene family display interwoven transcriptional and phenotypic roles in *Candida albicans*

Emily Simonton[1], Nayeli Cangelosi[2], Matthew Z. Anderson[2,3]

[1]Microbiology Doctoral Training Program, University of Wisconsin-Madison, USA. [2]Department of Medical Genetics, University of Wisconsin-Madison, USA. [3]Center for Genomic Science Innovation, University of Wisconsin-Madison, USA

Abstract

Gene duplication is the most common mechanism of producing new genes across the tree of life. Cells containing duplicated genes are usually culled from the population due to fitness defects. Thus, retained paralogs may have implications to organismal fitness by increasing gene expression, encoding a specialized function, or acquiring novel function. Repeated duplication can lead to the formation of gene families with high homology. While the function of gene duplicates has been studied in detail, little work has explored how the repeated emergence of paralogs may restrict the development of novel function or impact redundancy. Here, we constructed a panel of single deletion mutants for the *Candida albicans* telomere-associated (*TLO*) gene family to test for phenotypes caused by loss of just one of the 14 paralogs. Tlo proteins function as interchangeable subunits of the Mediator transcriptional regulatory complex and therefore have the potential to alter a wide range of phenotypes, including those linked to the balance between commensalism and pathogenesis. Transcriptional and phenotypic analysis suggested both redundant and non-redundant functions among individual paralogs. Most phenotypes were altered in at least one *TLO* mutant, such as for cell wall perturbation and lactate utilization. Conversely, most mutants were indistinguishable from the wildtype for growth rate in nutrient-rich medium and antifungal drug responses. Importantly, *TLO* double mutants produced synergistic phenotypes, suggesting that *TLO*s "talk" to each other. *TLO* retention appears to enable adaptation across diverse conditions, with each gene contributing to significant and widespread expression changes, irrespective of architectural group association.

BLOCK A

Phenotypic characterisation and functional genomics reveal mechanistic insights into Antifungal Drug Tolerance in Candida glabrata

SREYASHI ACHARJEE, Edel M.Hyland

Queens University Belfast, United Kingdom

Abstract

The rise in *Candida* infections, alongside increasing antifungal resistance (AFR) driven by genetic factors, is well-documented. However, recent studies by Berman et al. (2020) in *C. albicans* have identified a related yet distinct phenotype, antifungal drug tolerance (AFDT), where a subpopulation survives above the MIC of certain anti-fungal drugs, contributing to poor treatment outcomes and patient mortality. AFDT remains poorly studied in *C. glabrata*, prompting our research to characterize its conservation and understand its underlying mechanisms in another *Candida* species.

We quantified AFDT in six clinical isolates and a typed *C. glabrata* strain (ATCC2001) using disk diffusion and supra-MIC growth assays. Our findings underscore the significant variability in AFDT response to azoles and other environmental stressors among the tested isolates and the wild-type. We further identified strains exhibiting robust high or low-tolerant phenotypes , suggesting a strong genetic-environmental interplay. *In-vivo* experiments *in Galleria mellonella* revealed high-tolerant strains of *C. glabrata* are significantly difficult to eradicate than the low-tolerant strains. To elucidate the mechanisms underlying these ADFT phenotypes, we conducted an in-depth transcriptomic analysis on tolerant and non-tolerant states of three independent *C. glabrata* strains. This RNAseq analysis identified numerous metabolic pathways that are differentially regulated in tolerant versus non-tolerant states of the same strain. Furthermore, we uncovered critical genes that modulate the complex phenotypic differences between high and low-tolerant strains.

Overall, our findings provide critical insights into AFDT in *C. glabrata*, underscoring its complexity and clinical relevance. Continued research is essential to refine treatment strategies for *Candida* infections.

BLOCK A

Transcriptional Decoupling of Filamentation and Damage in *Candida albicans*

Siobhan Dietz[1], Norma Solis[2], Robert Fillinger[3], Scott Filler[4,5], Matthew Anderson[6,7]

[1]Cellular & Molecular Pathology Graduate Program, University of Wisconsin - Madison, USA. [2]Division of Infectious Diseases, Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, USA. [3]The Ohio State University, USA. [4]Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, USA. [5]David Geffen School of Medicine at UCLA, USA. [6]Department of Medical Genetics, University of Wisconsin – Madison, USA. [7]Center for Genomic Science Innovation, University of Wisconsin – Madison, USA

Abstract

The ability for *Candida albicans* to colonize, disseminate, and persist across diverse host niches is due, in part, to its transcriptional responsiveness to environmental cues. Two phenotypes critical for the transition from harmless colonization to virulence in the host are filamentation and epithelial damage, which facilitate expansion of *C. albicans* beyond colonized mucosal niches to produce disseminated disease. We investigated the genetic basis for opposing filamentation/damage phenotypes observed *in vivo* and *in vitro* between two strains, 529L and SC5314. 529L displays canonically "commensal" phenotypes in the host (non-filamentous/non-damaging), while SC5314 is considered to display "pathogen" phenotypes (filamentous/damaging). To define their differential responses to the host, we transcriptionally profiled each *C. albicans* strain incubated with oral epithelial cells along a time-course. RNA-sequencing revealed that despite massively different phenotypes, these strains display similar temporal patterns of gene expression during interaction with host cells. Strikingly, genes that regulate filamentation and their downstream effectors were expressed similarly in both strains. Additionally, members of a core filamentation network showed the same pattern of expression across the time course for both strains. The only functional class enriched in the strain SC5314 encompassed septal genes that are likely linked to forming hyphae. These data suggest that the lack of filamentation associated with commensalism in 529L is not due to altered transcriptional regulation but likely the result of a post-transcriptional or structural defects in hyphal formation. We are currently constructing mutants to test how regulation of septation genes between strains may lead to differential filamentation.

BLOCK A

Impact of vendor-specific bacterial and fungal variations in the murine gastrointestinal tract on the colonization with *Candida albicans*

Sarah Vielreicher[1], Wibke Böhnke[1], Cristina Zubiría-Barrera[2], Tilman Klassert[2], Katja Schubert[1], Tobias Köhler[1,3], Bianca Brisca[4], Rakesh Santhanam[5], Gianni Panagiotou[5], Megan Lenardon[4], Hortense Slevogt[2], Ilse Jacobsen[1]

[1]Leibniz Institute for Natural Product Research and Infection Biology, Microbial Immunology, Germany. [2]Helmholtz Centre for Infection Research, Dynamics of Respiratory Infections, Germany. [3]Julius-Maximilians-University Würzburg, Institute of Hygiene and Microbiology - Medical Mycology, Germany. [4]University of New South Wales, School of Biotechnology and Biomolecular Sciences, Australia. [5]Leibniz Institute for Natural Product Research and Infection Biology, Microbiome Dynamics, Germany

Abstract

Candida albicans interacts extensively with other members of the human gastrointestinal microbiota. These interactions play a crucial role in host health and disease, particularly by mediating colonization resistance exerted by resident bacteria against *C. albicans*. In this context, microbial dysbiosis due antibiotic treatment represents a major risk factor for the development of candidiasis.

To identify bacterial candidates involved in colonization resistance against *C. albicans*, we investigated natural microbiota variation in laboratory mice sourced from different breeding facilities. Fecal samples from 20 C57BL/6 colonies were subjected to 16S and ITS rRNA gene sequencing. Based on differences in taxonomic composition and microbial load, five colonies were selected for further colonization experiments. After antibiotic treatment and oral inoculation with *C. albicans*, fecal samples were collected at multiple time points to monitor fungal burden and changes in the intestinal microbiota composition.

Despite pre-existing differences in bacterial and fungal community composition across colonies, all five mouse cohorts exhibited similar patterns of *C. albicans* colonization. Antibiotic treatment led to the expected increase in fungal colonization. Surprisingly, sucrose supplementation in drinking water was sufficient to support stable and significant *C. albicans* colonization.

These results demonstrate that substantial microbiota variation in breeding colonies of laboratory mice does not necessarily influence colonization resistance. Nevertheless, fecal microbiome analysis revealed changes in bacterial composition associated with *C. albicans* colonization, with even stronger associations observed with housing conditions and sucrose supplementation through the experimental phase. This dataset will be utilized in further analyses to identify bacterial candidates responsible for colonization resistance.

BLOCK A

A genetic screen in Candida albicans for modulators of the neutrophil oxidative burst

Huan Jiang, Borko Amulic, Angela Nobbs

University of Bristol, United Kingdom

Abstract

Candida albicans (*C. albicans*) is an opportunistic fungal pathogen that poses serious threats to human health, especially in individuals with compromised immune systems. Neutrophils, as frontline immune responders, play a crucial role in controlling *C. albicans* infections through mechanisms such as oxidative burst, phagocytosis, degranulation and NETosis. However, *C. albicans* has evolved sophisticated immune evasion mechanisms to resist neutrophil-mediated killing, many of which remain uncharacterised. This project conducts a screen of the *C. albicans*GRACE (Gene Replacement and Conditional Expression) mutant library, using ROS production by human neutrophils as a readout. Our goal is to identify *C. albicans* factors that modulate the neutrophil response, either by (1) enabling recognition by neutrophils or (2) evading neutrophil activation. We screened 700 mutants (30% of the GRACE mutant library), and identified 14 potential hits, 6 of which mutants were selected for further investigation. By exploring the genetic basis of *C. albicans* resistance to neutrophils, our study will contributes to a better understanding of fungal pathogenesis and reveal pathways to enhance host immune efficacy. *C. albicans* proteins that modulate neutrophils' antifungal response represent potential therapeutic targets for this growing global threat.

BLOCK A

Epitranscriptomic Regulation of *Candida albicans* Pathogenicity by RNA Methylation

Anushka Nayak[1], Azam Khedri[1], Alejandro Chavarria[2,1], Kyla Sanders[3,1], Sohail Khoshnevis[1]

[1]Emory University, USA. [2]Northwestern University, USA. [3]Spelman College, USA

Abstract

Candida albicans, an opportunistic fungal pathogen, causes life-threatening infections in immunocompromised patients. Current antifungals are limited by toxicity, drug-drug interactions, and emerging resistance, underscoring the importance of identifying novel treatment approaches. The ability of *C. albicans* to invade deep tissues and organs for systemic infection is primarily attributed to the morphological transition of the pathogen from single budding yeast cells to hyphal filaments. In other eukaryotic cell types, mRNA modification has been identified as a key factor for various forms of cell differentiation, and N6-methyladenosine (m6A) is the most prevalent post-transcriptional modification of mRNA, related to mRNA stability, export, and decay. In yeast, Ime4 (ortholog of human METTL3) is an S-adenosylmethionine (SAM) dependent methyltransferase that functions as the only m6A 'writer.' Here, we determine that mRNA m6A levels, along with Ime4 methyltransferase expression levels, change during the morphological transition of *C. albicans*, and Ime4 knockdown inhibits hyphal formation. We also identified sinefungin, a small-molecule inhibitor that targets SAM-dependent MTases, including Ime4, to selectively disturb m6A formation in *C. albicans*. Our data indicate that submicromolar concentrations of sinefungin impair pathogenic traits of *C. albicans* including hyphal morphogenesis, biofilm formation, adhesion to epithelial cell lines, and virulence towards *Galleria mellonella*, highlighting sinefungin as an avenue for therapeutic intervention. Collectively, our data propose sinefungin as a potent molecule against *C. albicans* and emphasize further exploration of post-transcriptional control mechanisms of pathogenicity for antifungal design.

BLOCK A

Exploring the role of the CCAAT-binding complex in cell wall maintenance and biofilm formation in *Candida albicans*

Dinh-Dong Le[1], Wen-Han Wang[1], Chung-Yu Lan[1,2,3]

[1]Institute of Molecular and Cellular Biology, National Tsing Hua University, Hsinchu, Taiwan. [2]Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan. [3]School of Medicine, National Tsing Hua University, Hsinchu, Taiwan

Abstract

The *Candida albicans*Hap complex is a CCAAT-binding complex (CBC) known to regulate iron homeostasis through its association with another transcription factor, Hap43. Interestingly, diverse functions of the CBC independent of Hap43 have also been identified. In this study, we further explored new roles of the CBC and found that it is also involved in cell wall maintenance and biofilm formation in *C. albicans*. Moreover, while the small GTPase Rhb1 and the Mkc1 signaling pathway contribute to CBC-mediated maintenance of the cell wall, CBC-mediated biofilm formation appears to be independent of Rhb1. Finally, RNA sequencing (RNA-seq) and data analysis revealed functional divergence between the two Hap3 orthologs, Hap31 and Hap32. Notably, this work provides new insights into the broad influence of the CBC in *C. albicans*.

BLOCK A

Co-ordinated regulation of multi-drug transporters facilitates broad-spectrum antifungal resistance in *Clavispora* (*Candida*) *lusitaniae*

Dhanabala Subhiksha Rajesh Khanna[1], Carolina Piña Páez[2], Elora Demers[1], Kiran Mirpuri[1], Jason Stajich[2], Deborah Hogan[1]

[1]Dartmouth college, USA. [2]University of California Riverside, USA

Abstract

Infections caused by the emerging pathogenic yeast *Clavispora (Candida) lusitaniae* can be difficult to manage due to multi-drug resistance. Resistance to the frontline antifungal fluconazole (FLZ) in *Candida* spp. is commonly acquired through gain-of-function (GOF) mutations in the transcription factor gene *MRR1*. These activated Mrr1 variants enhance FLZ efflux via upregulation of the multi-drug transporter gene *MDR1*. Recently, it was reported that, unlike in *Candida albicans*, *C. lusitaniae* and *Candida parapsilosis* with activated Mrr1 also have high expression of another multi-drug transporter gene *CDR1* involved in FLZ resistance. To better understand the mechanisms of Mrr1 regulation of *MDR1* and *CDR1*, and other co-regulated genes, we performed CUT&RUN analysis of Mrr1 binding sites. Mrr1 bound the promoter regions of *MDR1* and *CDR1* as well as *FLU1*, which encodes another transporter capable of FLZ efflux. Mdr1 and Cdr1 independently contributed to the decreased susceptibility of the $MRR1^{GOF}$ strains against diverse clinical azoles and other antifungals including 5-flucytosine. A consensus motif, CGGAGWTAR, enriched in Mrr1-bound *C. lusitaniae* DNA was also conserved upstream of *MDR1* and *CDR1* across species including *C. albicans*. CUT&RUN and RNA-seq data were used to define the Mrr1 regulon which includes genes involved in transport, stress responses, and metabolism. The genetic regulation of two important multi-drug transporters, *MDR1* and *CDR1*, by a single regulator could be evolutionarily advantageous as a single nucleotide change favoring an $MRR1^{GOF}$ phenotype is sufficient to protect against a broad spectrum of antifungals and potentially aid secondary resistance acquisition in emerging *Candida*.

BLOCK A

Candida albicans Utilises Methaemoglobin to Form Hypervirulent, Drug resistant Polymicrobial Aggregate Biofilms

Campbell Gourlay, Ed Deshmukh-Reeves

University of Kent, United Kingdom

Abstract

The growth of drug resistant poly-microbial biofilms represents a major clinical problem that underpins recurrent infection and failed therapy. Here we show how the presence of Methaemoglobin, (MetHb), which forms when the iron component in haemoglobin is oxidised from the ferrous (Fe2+) to the ferric (Fe3+), state is bound by *C. albcians* into dense biofilms structures that show remarkable drug resistant properties. *C. albicans*/MetHb aggregate biofilms show increased virulence and a propensity to increases the rate of *S. aureus* and *P. aeruginosa* incorporation into poly-microbial biofilms. We provide a detailed account of the mechanisms that underpin *C. albicans*/MetHb interaction and their underlying properties. As MetHb levels are increased within a number of disease pathologies, including sepsis, *C. albicans*/MetHb aggregate biofilms may represent an as yet unexplored structure that supports metastatic infection and poor patient outcome.

BLOCK A

The Hst1–Sum1 repressor complex: Transcription regulation and DNA binding profile in the pathogenic yeast *Candida glabrata*

Bowen Liu, Laura Rusche

University at Buffalo, USA

Abstract

The SUM1 complex (SUM1C), composed of the histone deacetylase Hst1 and the transcription factor Sum1, represses a broad set of genes in *Candida glabrata*, including those involved in NAD^+ metabolism (*TNA1, TNR1, TNR2*) and antifungal resistance (*PDR1, CDR1*). CgSum1 lacks a DNA-binding domain found in *S. cerevisiae* Sum1, suggesting that SUM1C shifted to a different set of target genes during *C. glabrata* evolution, possibly contributing to its development into a pathogen.

To explore the rewiring of the SUM1C regulon, we conducted ChIP-seq to identify SUM1C binding sites and RNA-seq to examine transcriptional changes in the absence of SUM1C (HST1N293A and sum1Δ mutants). A list of genes associated with SUM1C by ChIP-seq and differentially expressed in RNA-seq are involved in meiotic processes, NAD^+ transport, and biofilm formation, suggesting a broader role for SUM1C in virulence.

Analysis of SUM1C-bound loci revealed a conserved sequence motif resembling a candidate transcription factor binding site from *S. cerevisiae*, raising the possibility that this transcription factor facilitates SUM1C recruitment in the absence of a DNA-binding domain in CgSum1. We added an HA tag to the transcription factor for ChIP and co-IP assays. We also deleted an instance of the motif to evaluate its role in SUM1C recruitment. Preliminary results are consistent with the transcription factor partnering with SUM1C.

This integrated approach will clarify how SUM1C is recruited to target genes and how the set of regulated genes differ from those in non-pathogenic relatives, offering insight into transcriptional rewiring during the evolution of *C. glabrata* pathogenicity.

BLOCK A

Manogepix treatment of *Candida albicans* alters immune recognition

Harry Osborne, Mark Stappers, Neil Gow

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

Abstract

Antifungal therapies have the potential to impact antifungal immunity and therefore may influence therapeutic outcome. Interactions between cells of the immune system and *Candida albicans* occur through pattern recognition receptors recognising pathogen associated molecular patterns (PAMPs), of which a majority are cell wall components. Several classes of current and novel antifungal chemotherapies target cell wall biosynthesis because wall integrity is essential for fungal growth and survival.

We investigated the immunological consequences of treatment of fungal pathogens with the novel glycosylphosphatidylinositol (GPI)-anchor biosynthesis inhibitor manogepix (MGX). We reasoned that the loss of outer cell wall mannoproteins, exposing the highly immunogenic inner cell wall components, may alter immune responses. Cell wall analyses indicated substantial alterations in response to drug treatment: outer cell wall mannan was significantly reduced as predicted from current cell wall models, and the inner cell wall polysaccharides β-1,3-glucan and chitin were exposed. However, soluble mannan and glucan detecting C-type lectin immune receptors demonstrated non-linear and receptor-specific changes in binding to inner and outer cell wall PAMPs. Human peripheral blood mononuclear cells challenged with *C. albicans* treated with sub-MIC_{50}MGX concentrations stimulated increased pro-inflammatory cytokine release, which diminished with increasing MGX concentrations. These findings indicate profound effects on the immune response due to antifungal treatment of *C. albicans.*

BLOCK A

Investigation of the role of RNA interference in *C. albicans*

Elise Iracane, Alessia Buscaino

University of Kent, United Kingdom

Abstract

RNA interference (RNAi) is a fundamental regulatory pathway with a wide range of functions, including regulation of gene expression and maintenance of genome stability.

It is widespread in the fungal kingdom (e.g. *Cryptococcus spp*., *Schizosaccharomyces pombe*), although it was lost in some species like *Saccharomyces cerevisiae.*Two main actors have been described to have a key role in the pathway: the exonuclease Dicer (for siRNA production) and the protein Argonaute (to bring siRNAs to their target).

We recently showed that *C. albicans* was RNAi efficient, however a small subset of clinical isolates, including the reference strain SC5314, has an inactive Argonaute protein due to a single SNP.

Working with several clinical isolates and the reference strain SC5314, we are using CRISPR-Cas9 gene editing and high-throughput small and long RNA sequencing to investigate the siRNA targets and therefore the role of RNAi in *C. albicans*. Additionally, we are exploring different conditions to identify the environments where RNAi is required.

BLOCK A

Chitin-glucan and chitosan as innovative solutions against candidiasis

Christel Rousseaux[1], Caroline Dubuquoy[1], Philippe Chavatte[2], Véronique Maquet[3], Valérie Gordenne[4], Salvatore Modica[4], Benoit Palms[4], Pierre Desreumaux[5]

[1]Intestinal Biotech Development, Lille, France. [2]U1286 - INFINITE - Institute for Translational Research in Inflammation, Univ. Lille, Inserm, CHU Lille, France. [3]KitoZyme SA. Parc Industriel des hauts Sarts Zone 2, Herstal, Belgium. [4]BiOkuris SA Parc Industriel des hauts Sarts Zone 2, Herstal, Belgium. [5]Hepato-Gastroenterology Department, CHU Lille, France

Abstract

Fungal chitin-glucan (CG) and chitosan (CS) are emerging antifungal agents capable of disrupting the *Candida albicans* (*C. albicans*) cell wall. As the gut is a primary reservoir for *C. albicans*, it may contribute to gastrointestinal and recurrent vulvovaginal candidiasis. This study assessed the antifungal properties of CG and/or CS through molecular modeling, *in vitro*, and *in vivo* models. In silico, docking and molecular dynamics simulations revealed that CG and CS chelate phospholipomannan (PLM), a key virulence factor of C. albicans, with a synergistic effect when combined. In vitro, mature Candida biofilms were exposed to CS (2.5–10 mg/mL) or amphotericin B (3–12 µg/mL). CS significantly reduced biofilm biomass in a dose-dependent manner (53%–91%), outperforming amphotericin B (18%–32%). *In vivo*, a DSS-induced colitis mouse model colonized with *C. albicans* was used to assess efficacy. Oral administration of CG+CS (1.5–3 g/day) was well tolerated and led to a marked reduction in fungal burden (from 245±190 to 0–9±6 cfu/100 mg stools), comparable to fluconazole (150 mg/kg/day). CG+CS also significantly improved disease activity index (DAI) scores (0.2–0.4 vs. 1.7 in controls), matching fluconazole efficacy. These findings suggest that CG and CS not only neutralize fungal toxins but also eradicate biofilms and reduce fungal load and inflammation *in vivo*. The preclinical results support the development of BK004, a CS-based vaginal gel, as a promising therapeutic candidate against candidiasis.

BLOCK A

Distinct mechanisms drive caspofungin induced β-1,3-glucan exposure in *Candida albicans* yeast versus hyphae

Mikayla Mangrum, Alexander Truchon, Lily Harper, Todd Reynolds

University of Tennessee, Knoxville, USA

Abstract

A major virulence factor of *Candida albicans* is the ability to switch between yeast and hyphal forms in response to environmental cues. Yeast-hyphal switching, in combination with successful evasion of the host immune system, greatly increases *C. albicans* ability to induce disease. Treatment with echinocandins inhibits the synthesis of β-1,3-glucan, a highly immunogenic cell wall polysaccharide, causing cell wall architectural changes including increased chitin deposition and β-1,3-glucan exposure. In yeast, caspofungin induced cell wall remodeling occurs via signaling through cell wall stress pathways, including the Mkc1 MAP kinase pathway and the calcium-calcineurin pathway. However, it is unknown whether similar genetic mechanisms drive caspofungin induced cell wall remodeling in hyphae. We show that contrary to yeast, the Mkc1 and calcineurin pathways are dispensable for increased β-1,3-glucan unmasking and chitin deposition during caspofungin treatment in hyphae. Surprisingly, the Rho1 GTPase impacts only hyphal-specific unmasking in response to caspofungin. Given the apparent differences in caspofungin driven unmasking between yeast and hyphae, we investigated their respective transcriptional responses to the drug using RNAseq. We observed a robust transcriptional response in caspofungin treated yeast that differed from that in treated hyphae. There was an enrichment of 73 differentially expressed cell wall genes in yeast form cells, but only 10 differentially expressed cell wall genes in hyphae. Of these, only 6 cell wall-related genes were shared between yeast and hyphae. We assessed the impact deletion of these genes had on caspofungin induced β-1,3-glucan exposure and chitin deposition in both forms, and this data will be presented.

BLOCK A

Antifungal resistance and persistence of Candida albicans in Catheter-Associated Urinary Tract Infections

Shane A. Cristy[1,2], Jennifer N. Walker[1,2], Michael C. Lorenz[1,2]

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

Abstract

Candida species are the second most common cause of Catheter-Associated Urinary Tract Infections (CAUTIs), behind *E. coli*. CAUTIs are biofilm-based infections and, while biofilms have been studied in oral, venous, and *in vitro* contexts, little is known about *Candida albicans* biofilm formation in the urinary environment. We tested mutants of nine previously identified biofilm transcriptional regulators in an *in vitro* assay using silicone urinary catheters. While some regulators defined in other conditions, like Tec1 and Brg1, are also required in the urine, others, like Bcr1 and Ndt80, are not, suggesting that there is substantial specialization of this complex regulatory network to specific environmental conditions. Transcriptional analysis confirmed a dramatic difference between urine and RPMI-based biofilms, with the urinary environment inducing cellular processes for xenobiotic efflux, as well as metal homeostasis. We next screened the Homann library to identify additional regulators in the urinary environment and identified *MRR1*, a gene previously implicated in drug efflux, as being necessary for biofilm development. This is partially dependent on the presence of urea, suggesting that urinary cues regulate drug sensitivity. We used a collection of CAUTI isolates to explore the effect of urine on drug resistance. We found that several of the clinical isolates were susceptible to fluconazole when grown in RPMI but were resistant when grown in a novel synthetic urine medium. This was not the case with caspofungin, which may inform treatment recommendations. In total, CAUTIs represent a unique infection context that need more attention.

BLOCK A

Elucidating novel epistatic interactions associated with *Candida albicans* echinocandin resistance

Anthony Hendriks[1], Lauren Wensing[1], Philippe Després[1,2,3], Meea Fogal[1], Rebecca Shapiro[1]

[1]University of Guelph, Canada. [2]Broad Institute, USA. [3]Brown University, USA

Abstract

Candida albicans is an opportunistic fungal pathogen capable of causing life-threatening invasive infections. Antifungal resistance remains a persistent obstacle in treating *C. albicans* infection. This holds true for the echinocandins, which are the most frequently administered class of antifungals against severe *C. albicans* infection. The development of echinocandin resistance is linked to single-point mutations in the *FKS1* gene, encoding the drug target, which can cause wide-ranging fitness trade-offs and reprogramming of cellular processes. Understanding the epistatic interactions that modulate echinocandin drug resistance is essential for identifying genetic vulnerabilities in resistant strains. Here, we used an echinocandin-resistant *C. albicans* mutant isogenic with a standard laboratory strain in combination with our lab's optimized, inducible CRISPR interference (CRISPRi) system to systematically probe genetic interactions influencing resistance. Using this platform, we validated known interactions between *FKS1* mutations and calcineurin signaling. We also tested a panel of candidate genes previously identified in pooled CRISPRi screens, revealing strain-specific genetic interactions that support the reorganization of regulatory networks in the resistant context. Finally, ongoing genome-wide CRISPRi screening of echinocandin-resistant *C. albicans* will identify additional interactions relevant to the resistance phenotype. These findings highlight the complex genetic circuitry surrounding echinocandin resistance and suggest that certain cellular pathways may be uniquely targetable in resistant strains. Ultimately, this work provides a foundation for uncovering genetic vulnerabilities in drug-resistant *C. albicans* and advances our understanding of how resistance rewires cellular networks to maintain survival under antifungal stress.

BLOCK B

Gel-free immunoproteomics as a useful tool for the identification of IgA-recognized Candida albicans antigens

Marina Álvaro-Moya, Alba M Blesa, Daniel Prieto, Isabel Cortés-Prieto, Elvira Román, Jesús Pla, Rebeca Alonso-Monge

Microbiology and Parasitology deparment, Faculty of Pharmacy, Universidad Complutense de Madrid, Spain

Abstract

Background: As a commensal of the human gastrointestinal tract, *Candida albicans* induces a humoral response directed mainly to hyphal forms. In this work, we have identified *C. albicans* targets of sIgAs obtained from a murine commensalism model by a gel-free immunoproteomics approach.

Methods: Specific anti-*Candida* sIgAs were purified from stools of mice colonized with *C. albicans* and incubated with the surfome and the secretome of *C. albicans* filaments. These complexes were immunoprecipitated with magnetic beads bound to anti-mouse IgA antibodies, and IgA-targets were identified by mass spectrometry**.**Results were validated by overexpressing some of the identified immunogens in a filament-deficient *flo8* mutant and confirmed by indirect immunofluorescence.

Results: The analyses of the identified proteins revealed that 40% were categorized as "uncharacterized" in the databases, with approximately 10% lacking a signal peptide. GO-enrichment analyses indicate that the samples were enhanced in the response to oxidative stress, protein folding, and ROS metabolic processes. Among the identified and validated immunogens, adhesins such as Als3, Als1, and Hwp1, and the chaperone Ssa2 stand out.

Conclusion: The immunoproteomic approach allowed the identification of *C. albicans* sIgAs targets. The intestinal humoral response is directed to diverse proteins, many of them remaining uncharacterized.

BLOCK B

Role of the transcription factor Wor2 in biofilm formation of *Candida auris*

Marine Louvet[1], Jizhou Li[1,2], Danielle Brandalise[1], Daniel Bachmann[1], Alix Coste[1,2], Dominique Sanglard[1], Frédéric Lamoth[1,2]

[1]Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Switzerland. [2]Infectious Diseases Service, Department of Medicine, Switzerland

Abstract

Background

Candida auris is an emerging fungal pathogen with the ability to develop antifungal resistance and to cause nosocomial outbreaks. *C. auris* is able to adhere to abiotic surfaces and form biofilms, which contributes to interhuman transmission and virulence. In this study, we assessed the role of the zinc cluster transcription factor Wor2 (B9J08_002136) in biofilm formation of *C. auris*.

Methods

Two *WOR2* mutants were constructed in the wild-type strain IV.1 : *WOR2*^{HA} (hyperactivation of *WOR2*) and *wor2*Δ (deletion of *WOR2*). Phenotypic growth of the mutants was assessed, and their biofilm forming capacity was measured by crystal violet assays.

The downstream effectors of Wor2 were investigated by RNA sequencing, comparing *WOR2*^{HA} to IV.1. The function of these genes was further examined through their overexpression under the control of *ADH1* promoter at their native loci.

Results

The mutants did not exhibit any growth defect. Compared to the IV.1 strain, *WOR2*^{HA} reduced biofilm formation while *wor2*Δ increased biofilm formation. Transcriptomic analyses revealed significant downregulation of *ALS4112* and *SCF1* (two important adhesins) in *WOR2*^{HA}. Overexpression of *ALS4112* and *SCF1* in the IV.1 background showed increased biofilm production. In the *WOR2*^{HA} background, overexpression of *SCF1* could restore biofilm capacity, which was not the case for *ALS4112* overexpression.

Conclusion

This study demonstrates for the first time that Wor2 acts as repressor of biofilm formation in *C. auris*, probably by down regulation of Als4112 and Scf1, with Scf1 being the main downstream effector.

BLOCK B

Distinct Ire1-driven transcriptional responses control filamentous growth in *Candida albicans*

Samuel Stack-Couture[1], Vanessa Dumeaux[1], Rebecca Shapiro[2], Patrick Lajoie[1]

[1]Western University, Canada. [2]University of Guelph, Canada

Abstract

The Unfolded Protein Response (UPR) has been shown to regulate drug resistance and pathogenicity in *Candida albicans*. The UPR maintains secretory protein homeostasis in the endoplasmic reticulum (ER) by monitoring misfolded protein levels. When ER stress occurs, the sensor protein Ire1 detects stress and initiates the UPR by cleaving *HAC1*mRNA, enabling translation of the Hac1 transcription factor. Hac1 then activates multiple genes involved in ER quality control to restore cellular homeostasis.

While the UPR has been extensively studied in model yeasts like *Saccharomyces cerevisiae*, its function in fungal pathogens remains poorly understood. *C. albicans*, a significant pathogen causing invasive infections in immunocompromised patients, shows increased sensitivity to antifungal drugs when lacking a functional UPR. These UPR-deficient cells exhibit reduced pathogenicity, including inability to undergo filamentous growth—a critical virulence trait. However, the specific UPR target genes responsible for these phenotypes remain unidentified.

Using RNA sequencing, we analyzed transcriptomes of wild-type and *ire1DX* mutant cells (with diminished IRE1 expression) following treatment with ER stress-inducer tunicamycin or filamentation-inducing serum. Surprisingly, we found minimal overlap between responses to proteotoxic stress and filamentous growth induction. Furthermore, *C. albicans* shows only partial dependence on Hac1 during morphogenesis, suggesting Ire1 has Hac1-independent functions in this process. Through this work, we've identified novel pathways involved in *C. albicans* morphogenesis. Importantly, Ire1 has proven to be a druggable target in mammalian models, suggesting it could potentially serve as a therapeutic target for treating fungal infections. This discovery opens new avenues for developing antifungal therapies targeting the UPR pathway.

BLOCK B

Unveiling Novel Mechanisms of Fluconazole Resistance in *Candida auris*

Tracy L. Peters[1], Nicholas C. Cauldron[2], Katherine S. Barker[1], Qing Zhang[1], Christina A. Cuomo[2], P. David Rogers[1]

[1]St. Jude Children's Research Hospital, USA. [2]Brown University, USA

Abstract

Background:

Up to 90% of *Candida auris* clinical isolates are resistant to fluconazole. While azole resistance has been attributed to mutations in *ERG11* or *TAC1b* or increased *CDR1* expression, these do not account for fluconazole resistance in all clinical isolates. We aim to identify novel mechanisms of resistance by evolving *C. auris* strains in fluconazole.

Methods:

Two *C. auris* strains with *CDR1* disrupted were evolved in fluconazole. For each sample, the minimum inhibitory concentration (MIC) was determined using a modified CLSI broth microdilution assay and the genomes were sequenced via Illumina NovaSeq. CRISPR-Cas9 gene-editing was used to introduce selected mutations into a fluconazole-susceptible strain and azole MICs were determined. Available genotypes of clinical isolates were compared to in vitro populations.

Results:

Seventy-nine of the 96 evolved samples had a 2- to 64-fold increase in fluconazole MIC above the parental strain. Genome sequencing of strains with elevated MICs identified mutations associated with increased fluconazole resistance. While no mutations were observed in *ERG11* or *TAC1B*, mutations were discovered in 33 genes. Mutations in the genes encoding *CAP1*, *MRR1a*, and B9J08_004718 were present in samples with MICs ≥ 64 μg/mL. Introduction of amino acid substitution E403* in *Cap1*, G463S in *Mrr1*, or E688* in B9J08_004718 resulted in elevated fluconazole MICs of 16 μg/mL, 4 μg/mL, or 4 μg/mL, respectively. Similar variants in these genes were observed in fluconazole-resistant clinical isolates.

Conclusions:

These findings reveal *CDR1*-independent resistance determinants in *C. auris* that may be operative in resistant clinical isolates.

BLOCK B

Genome-wide association of copy number variation in clinical Candida auris links copy number variations to triazole resistance

Nicholas C. Cauldron[1], P. David Rogers[2], Christina A. Cuomo[1]

[1]Department of Molecular Microbiology and Immunology, Brown University, USA.
[2]Department of Pharmacy and Pharmaceutical Sciences, St. Jude Children's Research Hospital, USA

Abstract

Triazoles are the most utilized class of antifungal drug, accounting for more than 80% of antifungal prescribing in the United States. Multiple single nucleotide polymorphisms in *ERG11*, the target of these drugs, decrease triazole susceptibility in clinical populations of the emerging nosocomial pathogen *Candida auris*, however they do not completely explain highly elevated resistance phenotypes. Since copy number variation (CNV) including *ERG11* has been demonstrated to decrease triazole susceptibility in other *Candida* species, we hypothesized an unbiased approach would reveal candidate genes impacted by CNVs which contribute to triazole resistance in clinical *C. auris* populations. To test this hypothesis, we performed a genome-wide association study (GWAS) on approximately 900 *C. auris* genomes that could be matched with drug susceptibility data (minimum inhibitory concentrations (MICs) to fluconazole, combined from multiple sources). In the GWAS, we correlated gene deletions and duplications with changes in fluconazole MIC. The most significant duplicated gene was *ERG11*, which was found in clades I and III, and rarely in clade IV. There were no isolates with a CNV impacting *ERG11* that were susceptible to fluconazole. Six genes impacted by deletions showed high significance, including two encoding GPI-anchored proteins with no homologs outside of *C. auris* and two encoding transporters. Further analysis will interrogate the functional role of these candidates in triazole resistance of this important emerging pathogen.

BLOCK B

Uncovering actionable trade-offs of antifungal resistance in Nakasomyces glabratus (Candida glabrata)

Juan Carlos Nunez-Rodriguez[1,2], Miquel Àngel Schikora-Tamarit[1,2], Toni Gabaldón[1,2,3,4]

[1]Institure for Research in Biomedicine of Barcelona (IRB Barcelona), Spain. [2]Barcelona Supercomputing Center (BSC-CNS), Spain. [3]Catalan Institution for Research and Advanced Studies (ICREA), Spain. [4]Centro de Investigación Biomédica En Red de Enfermedades Infecciosas (CIBERINFEC), Spain

Abstract

The increasing prevalence of drug resistance represents a major clinical challenge considered an inevitable outcome of sustained exposure to antifungal drugs. To explore potential new therapeutic avenues, we investigated fitness trade-offs associated with azole and echinocandin resistance in *Nakaseomyces glabratus* (syn. *Candida glabrata*), a priority yeast pathogen showing growing incidence of drug and multidrug resistance. For this, we comprehensively phenotyped a large collection of azole- and echinocandin-resistant strains to uncover resistance-associated stress sensitivity trade-offs. Our results show that increased stress sensitivity is a common trade-off of drug resistance, with 98% of resistant strains exhibiting reduced fitness under at least one of the tested stresses. Despite the diversity of genetic backgrounds and resistance mechanisms, we identified consistent trends in some resistance-associated vulnerabilities. Multivariate modelling revealed complex genetic interactions underlying these trade-offs. Importantly, we experimentally validated the potential of targeting these fitness trade-offs. Cyclosporin A selectively inhibited anidulafungin-resistant strains, while NaCl effectively suppressed the emergence of fluconazole resistance. This study highlights the widespread occurrence of fitness costs associated with antifungal resistance and emphasizes their potential as a novel therapeutic strategy to combat this growing threat.

BLOCK B

Identifying regulators of aneuploidy and their effects on antifungal drug resistance in Candida albicans

Philipp Brandt[1,2], Eli I. Maciel[2], Cécile Gautier[2], Erik Maikranz[3,4], Andrey Aristov[3], Corinne Maufrais[1,2,5], Charles Baroud[3,4], Christophe d'Enfert[1], Mélanie Legrand[1], Iuliana V. Ene[2]

[1]Institut Pasteur, Université Paris Cité, Fungal Biology and Pathogenicity Unit, INRAe USC2019, Paris, France. [2]Institut Pasteur, Université Paris Cité, Fungal Heterogeneity Group, Paris, France. [3]Institut Pasteur, Université Paris Cité, Physical Microfluidics and Bioengineering Unit, Paris, France. [4]LadHyX, CNRS, Ecole Polytechnique, Institut Polytechnique de Paris, France. [5]Institut Pasteur Bioinformatic Hub, Université Paris Cité, Paris, France

Abstract

Candida albicans causes an estimated 1.5 million disseminated infections and possibly up to 1 million deaths annually. During antifungal therapy, a subset of fungal isolates (10–49%) can persist in the bloodstream despite being drug-susceptible *in vitro*, suggesting non-conventional resistance mechanisms. We have shown that exposure to high azole concentrations can induce heteroresistance, a reversible drug-resistant state, linked to increased chromosome copy numbers. This suggests a connection between aneuploidy and azole heteroresistance. However, the mechanisms behind aneuploidy formation in *C. albicans* remain unclear.

To identify genes regulating aneuploidy, a *C. albicans* overexpression mutant collection (ORFeome collection) is being screened for ploidy changes using DNA staining and flow cytometry. This collection includes ~4,500 mutants covering ~75% of the protein-coding gene repertoire. Half of the library has been screened for ploidy changes, revealing three groups: (1) near-diploid (91%), (2) decreased ploidy (7.6%), and (3) increased ploidy (1.4%). Genes whose overexpression increased chromosome copy numbers encode transcription factors, protein kinases, putative GPI-anchored proteins and several uncharacterized genes. Decreased ploidy mutants include those for chromatin remodelers, a putative lipase and several uncharacterized genes.

To study aneuploidy dynamics, we use a BFP/GFP fluorescence reporter system integrated on a specific chromosome of interest, combined with microfluidics and flow cytometry. This allows real-time tracking of chromosome changes and identification of features linked to the emergence of aneuploid cells. These insights will advance our understanding of azole heteroresistance and uncover novel regulators of aneuploidy in *C. albicans*.

BLOCK B

MiniChIP-MS: a novel approach to identify transcription factors assembling on promoters in *Candida* spp.

Teera Leepattarakit, David Teixeira, Elizabeth Hughes, Takashi Kubota

University of Aberdeen, United Kingdom

Abstract

In *Candida* spp, transcriptional regulation plays a crucial role in acquiring drug resistance and controlling virulence. However, it has not been fully understood yet, particularly in *Candida* (*Candidozyma*) *auris*.

We are successfully developing a novel approach, minichromosome immunoprecipitation followed by mass spectrometry (MiniChIP-MS), to identify transcription factors assembling on promoter of interest in *Candida* spp. Previously, we have developed MiniChIP-MS in *Saccharomyces cerevisiae* for analysing promoter of the *PDR5* multidrug transporter gene (Nikolov et al. 2022 Nature Communications). Here, we successfully improved MiniChIP for *S. cerevisiae* and now can detect transcription factors (Pdr1, Pdr3 and Ume6) and their cofactors including Mediator, SWI/SNF, SAGA, TFIID, and Rpd3S on the *PDR5* promoter. We are further applying this MiniChIP for promoters of *ERG* genes in *S. cerevisiae* to reveal dynamic regulation of ergosterol synthesis genes.

We are currently applying MiniChIP for *Candida* spp, *Candida glabrata* (*Nakaseomyces glabratus*) and *C. auris*. For *C. auris*, we are investigating proteins assembled on promoter of the *CDR1* multidrug transporter gene by MiniChIP-MS and identified transcription factors Tac1a and Tac1b as well as other transcription factors. Furthermore, we are utilising this MiniChIP-MS for analysing promoter of an adhesion gene in *C. auris* to identify transcription factors regulating its expression.

MiniChIP-MS is a robust approach to identify transcription factors on promoter of interest and can be utilised for any promoters and potentially any DNA regions of interest in *Candida* spp.

BLOCK B

Complex mechanisms underlying the effects of extracellular vesicles released by health-promoting bacteria on *Candida* virulence and biofilm formation

Justyna Karkowska-Kuleta[1], Michalina Mical[1], Katarzyna Kowalik[1,2], Kamila Kulig[1], Elzbieta Rudolphi-Szydlo[3], Anna Barbasz[3], Maria Rapala-Kozik[1]

[1]Department of Comparative Biochemistry and Bioanalytics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland. [2]Doctoral School of Exact and Natural Sciences, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland. [3]Department of Biochemistry and Biophysics, Institute of Biology and Earth Sciences, University of the National Education Commission, Krakow, Poland

Abstract

Different microbial species coexist during host colonization, exhibiting either cooperative or competitive interactions that influence the dynamics of invasion and adaptation. Within the complex interplay of microbial communities, extracellular vesicles (EVs)—nanometer-sized structures with a lipid bilayer and diverse cargo—produced by cohabiting bacteria and fungi may play a key role in cross-kingdom communication.

Considering the well-documented antifungal and anti-biofilm effects of health-promoting bifidobacteria and lactobacilli, or their metabolites, we investigated the potential role of EVs derived from selected probiotic species in controlling *Candida albicans* and non-*albicans Candida* viability and biofilm formation capacity. Specifically, the impact of bacterial EVs on biofilm thickness, detachment, metabolic activity of biofilm-forming cells, their filamentation, membrane stability and cell survivability across different biofilm developmental stages—as well as within mature biofilms—was examined for *C. albicans*, *C. tropicalis*, and *C. parapsilosis*.

Despite the probiotic nature of all tested bacterial strains, their EVs exhibited an unexpected dual effect—while destroying some fungal cells, they also significantly stimulated fungal proliferation, hyphal formation, and biofilm growth. Furthermore, the presence of EVs exerted a differential impact on fungal cell metabolism, markedly inhibiting mitochondrial enzyme activity while, in some cases, promoting ATP production. We also demonstrated that the complex interactions between fungal cells and bacterial vesicles may be triggered by EVs' initial binding to surface adhesins—*C. albicans* Als3 and *C. tropicalis* Hyr1. In summary, it can be inferred that *Candida* fungi may benefit from EVs produced by probiotic bacteria.

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BLOCK B

Investigating the role of chromatin modifiers in Candida albicans morphogenesis

Nelson Mounguengue, Natacha Sertour, Sophie Bachellier - Bassi, Christophe D'Enfert

Institut Pasteur, France

Abstract

The yeast *Candida albicans* is a benign member of the human gut microbiota that can turn into one of the deadliest human opportunistic fungal pathogens. A distinctive feature of *C. albicans* is its ability to adopt different morphologies in response to environmental variations. These different phenotypic forms -unicellular budding yeast, filamentous pseudohyphal and hyphal forms or the more elusive chlamydospores- are genetically identical.

Chlamydospores appear *in vitro* when *C. albicans* faces adverse growth conditions: a rather low temperature (25°C), microaerophilia and nutrient-poor medium. To date, little is known about the mechanisms involved in chlamydospore development, although our lab has demonstrated that this differentiation is under a tight transcriptional control.

Here, we have investigated the role of chromatin modifiers in the formation of *C. albicans* chlamydospores. First, we have established a novel method combining enzymatic treatment and flow cytometry to purify and quantify chlamydospores. This method allows measuring the effect of mutations on chlamydospore formation efficiency and consequently the comparison of chlamydosporulation across different strains. Second, taking advantage of this method, we have shown that histone deacetylases (HDACs) play a key role in chlamydospore formation. Indeed, we have highlighted the role of three *C. albicans* HDACs (Hda1, Hos2 and Rpd31) in the dynamics of chlamydospore formation. Furthermore, we have shown the involvement of other chromatin modifiers such as the acetyl transferase Gcn5 in chlamydosporulation.

In summary, our work has demonstrated that epigenetic regulation plays a major role in chlamydospore formation; the regulation network of this phenomenon is currently being investigated.

BLOCK B

The Community State Type I dominated by *Lactobacillus crispatus* is the most effective vaginal microbiota in counteracting *Candida albicans* infection *in vitro*

Luca Spaggiari[1], Natalia Pedretti[2], Samyr Kenno[3], Andrea Ardizzoni[3], Giuseppina Campisciano[2,4], Karin Sossi[4], Nunzia Zanotta[4], Ramona Iseppi[5], Carla Sabia[5], Patrizia Messi[5], Francesco De Seta[6,2], Manola Comar[2,4], Robert Wheeler[7,8], Eva Pericolini[3]

[1]Clinical and Experimental Medicine Ph.D. Program, University of Modena and Reggio Emilia, Modena, Italy. [2]Department of Medicine, Surgery and Health Sciences, University of Trieste, Trieste, Italy. [3]Department of Surgical, Medical, Dental and Morphological Sciences with interest in Transplant, Oncological and Regenerative Medicine, University of Modena and Reggio Emilia, Modena, Italy. [4]Department of Advanced Translational Microbiology, Institute for Maternal and Child Health - IRCCS Burlo Garofolo, Trieste, Italy. [5]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy. [6]Department of Obstetrics and Gynaecology, IRCCS San Raffaele Scientific Institute, University Vita and Salute, Milan, Italy. [7]Department of Molecular and Biomedical Sciences, University of Maine, Orono, Maine, USA. [8]Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, Maine, USA

Abstract

The lactobacilli-dominated vaginal microbiota plays a key role in protecting the host against fungal pathogens such as *Candida (C.) albicans*. In this work, we developed four artificial human vaginal microbiotas reproducing the major *Lactobacillus*-dominated Community State Types (CSTs) found in healthy women as identified by Ravel and co-workers. Among these, the artificial CST I (aCST I), dominated by *Lactobacillus crispatus*, showed remarkable protective effects in an *in vitro* model of *C. albicans* infection. Co-infection with aCST I significantly reduced epithelial damage induced by the reference *C. albicans* strain and three clinical isolates, with protection entirely attributable to *L. crispatus*. Transcriptomic analyses revealed that *L. crispatus* strongly suppressed fungal virulence by downregulating key genes involved in hyphal growth, adhesion, and pathogenicity (e.g., *ECE1*, *ALS3*, *HWP1*, *HYR1*). It also induced an iron starvation response in *C. albicans*, marked by upregulation of iron acquisition genes and downregulation of *ALS3*, a ferritin-binding protein, alongside inhibition of fungal cell cycle progression. Moreover, presence of the aCST I microbiota modulated host immunity, reducing IL-1 receptor antagonist (IL-1RA) and β-defensin-2 levels during infection. Comparative analysis with human-derived CST I confirmed the model's translational validity. In contrast, aCST III, dominated by *L. iners*, enhanced fungal adhesion and failed to protect, while aCST II and aCST V showed only partial effects. Overall, our study highlights *L. crispatus* as a potent antifungal and immunomodulatory agent and supports the use of synthetic vaginal microbiotas. These findings could advance our understanding of host-microbe-pathogen interactions and developing microbiota-based interventions against fungal vaginal infections.

BLOCK B

The role of the central carbon metabolism of *Candida albicans* during superficial infections

Stefanie Wijnants, Odessa Van Goethem, Patrick Van Dijck

KU Leuven, Belgium

Abstract

Candida albicans is an opportunistic human fungal pathogen that relies upon various virulence traits to establish superficial epithelial infections. These include morphogenesis, adhesion, invasion, nutrient acquisition and metabolic adaptation. The central carbon metabolism plays a critical role in the virulence of *C. albicans*. Glycolysis is upregulated during systemic infections, while the alternative carbon metabolism supports survival within immune cells. Moreover, metabolic flexibility enhances virulence by dampening the host immune response. Notably, genes involved in the glyoxylate cycle and gluconeogenesis are upregulated during tongue infections in murine models. Despite these insights, the specific contributions of central carbon metabolic pathways of *C. albicans* to epithelial infections remain poorly understood. To address this knowledge gap, we generated mutant *C. albicans* strains with blocked central carbon metabolic pathways, including glycolysis, N-acetylglucosamine (GlcNAc) metabolism, β-oxidation, the glyoxylate cycle, and gluconeogenesis. These mutants were assessed for their virulence potential against oral, intestinal, and vaginal epithelial cells. Our results demonstrate that glycolysis is essential for full cytotoxicity towards epithelial cells. Additionally, both glycolytic and the alternative carbon metabolic pathways are crucial for efficient adhesion to the host cells. Finally, *In vivo* mouse models further confirmed that disruption of the central carbon metabolism significantly reduces virulence. These findings highlight the importance of metabolic adaptability and efficient carbon utilization in supporting *C. albicans* virulence during superficial epithelial infections in different host niches.

BLOCK B

More Than a Scissor: The Geh1 Glucanase as a Cell Wall Remodeler in *Candida*

Laura Alejandra RIVAS ARENAS[1], Clara BEKIRIAN[1], Christophe d'ENFERT[1], Sophie BACHELLIER-BASSI[1], Murielle CHAUVEL[1], Lionel BALLUT[2], Thierry FONTAINE[1]

[1]Institut Pasteur, France. [2]Institut de Biologie et Chimie des Protéins, France

Abstract

Candida albicans is one of the most threatening fungal pathogens. A key feature contributing to its adaptability and pathogenicity is its cell wall. While cell wall polymers such as chitin, β-1,3-glucan, and mannans have been explored extensively, β-1,6-glucan, which plays a critical role in the formation of the cell wall bilayer, remains relatively understudied, and its biosynthetic pathway is unknown. We have recently identified a novel hydrolase activity specific to β-1,6-glucan in *C. albicans*. This enzyme, named Geh1, exhibits both endo-β-1,6-glucanase and transglucosidase activities. Hydrolytic activity was initially detected only in the presence of glycerol, which functions as an acceptor. The production of the recombinant protein enabled detailed characterization, confirming the requirement for an acceptor and demonstrating that β-1,6-glucan oligomers can also serve this role. Deletion of *GEH1* did not produce a distinct phenotype or alter β-1,6-glucan content but its overexpression increased its levels. Structurally, the enzyme comprises two domains: a catalytic domain and a small auxiliary domain located at the C-terminus. The removal of this later domain abolishes the enzyme activity. Protein sequence alignments identified homologous enzymes in other *Candida* species, including *C. auris* and *C. glabrata*. Based on activity assays, *C. auris* Geh1 appears to behave similarly to *C. albicans* Geh1. However, in *C. glabrata*, the enzyme does not require glycerol as an acceptor. These findings suggest that Geh1 is not involved in β-1,6-glucan biosynthesis, but likely participates in cell wall remodeling, representing the first characterized fungal β-1,6-glucan transglucosidase.

BLOCK B

Mannan is a context-dependent shield that modifies virulence in *Nakaseomyces glabratus*

Gabriela Ribeiro[1], Emily Priest[2], Helen Heaney[3], Jonathan Richardson[2], Delma Childers[3]

[1]University of Guelph, Canada. [2]King's College London, United Kingdom. [3]University of Aberdeen, United Kingdom

Abstract

Fungal-host interaction outcomes are influenced by how the host recognizes fungal cell wall components. Mannan, a key cell wall carbohydrate, can serve as a glycoshield that masks inner β-1,3-glucan, preventing pro-inflammatory immune activation. Disturbing this glycoshield in *Candida albicans* results in enhanced antifungal host responses and reduced fungal virulence. However, deletions affecting mannan synthesis can lead to systemic hypervirulence for *Nakaseomyces glabratus* (formerly *Candida glabrata*), suggesting that proper mannan architecture dampens virulence for this organism. *N. glabratus* is the second leading cause of invasive and superficial candidiasis, but little is known about how the cell wall affects its pathogenesis. In order to better understand the importance of these species-specific cell wall adaptations in infection, we set out to investigate how the mannan polymerase II complex gene, *MNN10*, contributes to *N. glabratus* cell wall architecture, immune recognition, and virulence in reference strains BG2 and CBS138. *mnn10*Δ cells had thinner inner and outer cell wall layers and elevated mannan, chitin, and β-1,3-glucan exposure compared to wild-type cells. Consistent with these observations, *mnn10*Δ cells activated the β-1,3-glucan receptor in oral epithelial cells (OECs), EphA2, and caused less OEC damage than wild-type. *mnn10*Δ replication was also restricted in macrophages compared to wild-type controls. Yet, during systemic infection in *Galleria mellonella* larvae, *mnn10*Δ cells induced rapid larval melanization and BG2 *mnn10*Δ cells killed larvae significantly faster than wild-type. Thus, our data suggest that mannan plays context-dependent roles in *N. glabratus* pathogenesis, acting as a glycoshield in superficial disease models and modulating virulence during systemic infection.

BLOCK B

Elucidating Cross-Kingdom Interactions During Catheter-Associated Urinary Tract Infections

Ellsa Wongso, Dr. Ana Flores-Mireles, Dr. Felipe Santiago-Tirado

University of Notre Dame, USA

Abstract

Catheter-associated Urinary Tract Infections (CAUTIs) are one of the most prevalent hospital-acquired infections. Recent studies have estimated that during long-term catheterization, 81.5% of CAUTI involve three or more microbial species. It is also known that gram negative *E. coli*, gram positive *E. faecalis*, and fungal pathogen *C. albicans* are amongst the three most common pathogens found during CAUTI. These pathogens co-occur in the clinic, forming complex biofilms that protect them against antibiotic treatment and promote antibiotic resistance. Different microbial combinations were tested on *in vitro* biofilms that recapitulate the catheterized bladder environment. Our preliminary data show that growth and adherence of *C. albicans* on biofilms are reduced when incubated with *E. coli* but not *E. faecalis*. *E. coli* also reduces *C. albicans'* hyphal formation in human urine. RNA-sequencing suggests that changes in the expression of iron uptake and utilization genes play a role in the failure of *C. albicans* to maintain hyphae. In our *in vivo* study, *E. faecalis* co-localizes with *C. albicans* while *E. coli* and *C. albicans* are more likely to occupy different niches within the bladder during infections. *E. faecalis* promotes hyphal formation of *C. albicans* in the presence of *E. coli* as well as reduces killing of *C. albicans in vitro. In vivo*, *E. faecalis* facilitates *E. coli* and *C. albicans* co-localization, counteracting their antagonism. Notably, *E. coli* secretes small (<3 kDa) proteins that damage the *C. albicans* cell wall that is mitigated by the presence of *E. faecalis*, suggesting a novel interspecies interaction in CAUTI.

BLOCK B

Vaginal clinical isolates of *Candida albicans* differentially modulate type I interferon pathway in an *in vitro* infection model

Natalia Pedretti[1], Samyr Kenno[2], Luca Spaggiari[3], Andrea Ardizzoni[2], Manola Comar[1], Samuele Peppoloni[2], Robert T. Wheeler[4], Eva Pericolini[2]

[1]Department of Clinical, Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy., Italy. [2]Department of Surgical, Medical, Dental and Morphological Sciences with Interest in Transplant, Oncological and Regenerative Medicine, University of Modena and Reggio Emilia, Modena, Italy., Italy. [3]Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia Modena., Italy. [4]Department of Molecular and Biomedical Sciences, University of Maine, Orono, Maine, USA., USA

Abstract

Vulvovaginal candidiasis (VVC) affects up to 75% of women worldwide and is primarily caused by *Candida albicans* (*C. albicans*). Our studies demonstrated that two clinical isolates of *C. albicans*, a VVC-associated strain (Ca01887) and a Colonizing strain (Ca14314), elicit different epithelial responses. Ca01887 caused massive epithelial damage, elevated fungal shedding and exfoliation, without activating type I interferon (IFN-I) signaling. In contrast, Ca14314 induced limited damage and shedding and activated the IFN-I pathway.

Using an *in vitro* infection model with A-431 vaginal epithelial cells (VECs), we analyzed the IFN-I pathway's role. VECs were treated with a neutralizing IFNAR-2 antibody, the STING agonist 2'3'-cGAMP, and the JAK-STAT inhibitor Ruxolitinib, alone and in combination. After 24 hours, fungal shedding was quantified via colony-forming units (CFUs).

To assess inflammasome activation, IL-1β, IL-1α, and IL-1RA levels were measured in VECs supernatants using ELISA. Mitochondrial ROS (mtROS) production was monitored through MitoSOX Red staining and quantified by fluorometry and confocal microscopy. To correlate mtROS production with fungal shedding, cells were treated with the mtROS inhibitor Visomitin.

Our results showed that fungal shedding increased with Ruxolitinib and decreased with 2'3'-cGAMP in Ca14314-infected VECs, but not with Ca01887. Ca14314 induced significantly higher mtROS than Ca01887, while Ca01887 triggered a stronger inflammasome. Visomitin enhanced shedding only in Ca14314-infected VECs.

These findings reinforce the idea that IFN-I signaling and mtROS are key regulators in the vaginal epithelial cell defense to *C. albicans* infection, suggesting thus their possible employment as novel therapeutic targets in VVC/RVVC.

BLOCK B

Mutations in *TAC1B* drive increased *CDR1* and *MDR1* expression and fluconazole resistance in *C. auris*

Katherine Barker[1], Darian Santana[1], Brooke Esquivel[2], Qing Zhang[1], Tracy Peters[1], Jeffrey Rybak[1], Theodore White[2], Joachim Morschhäuser[3], Christina Cuomo[4], David Rogers[1]

[1]St. Jude Children's Research Hospital, USA. [2]University of Missouri-Kansas City, USA. [3]Universität Würzburg, Germany. [4]Brown University, USA

Abstract

Background: *Candida auris* has emerged as a critical priority pathogen partly due to its propensity to exhibit antifungal resistance, especially to the commonly prescribed antifungal fluconazole. A mutation in *TAC1B*, which encodes a zinc-cluster transcription factor, has been shown to confer increased resistance to fluconazole.

Methods: Strains harboring A640V, A657V, or F862_N866del *TAC1B* mutations found in clinical isolates, as well as their *cdr1-*, *mdr1-*, and *cdr1*/*mdr1*-disruptant derivatives, were constructed by CRISPR-Cas9 gene editing in strain 1c, a *TAC1B*^{WT}/*ERG11*^{WT} fluconazole-susceptible derivative of clinical isolate Kw2999. RNA sequencing was performed using Illumina NextSeq for stranded mRNA. Minimum inhibitory concentrations (MIC) were measured using modified CLSI methodology. Rhodamine 6G assays were employed to measure efflux, and ^{3}H-fluconazole uptake assays were employed to measure fluconazole import.

Results: RNA-seq of strains harboring A640V, A657V, or F862_N866del *TAC1B* mutations revealed that the ATP-Binding Cassette transporter gene *CDR1* as well as the Major Facilitator Superfamily transporter gene *MDR1* were both upregulated in the *TAC1B* mutant strains. All *TAC1B* mutant strains result in increased resistance to fluconazole and other triazole antifungals. *CDR1* disruption increased susceptibility in *TAC1B* mutant strains whereas disruption of *MDR1* had little to no effect. However, disruption of both *CDR1* and *MDR1* resulted in an additional increase in susceptibility as compared to *CDR1* disruption alone. *CDR1* disruption in clinical isolate Kw2999 (*TAC1B*^{A640V}/*ERG11*^{K143R}) resulted in reduced Rhodamine 6G efflux and increased import of ^{3}H-fluconazole whereas disruption of *MDR1* alone did not.

Conclusion: *CDR1* is the primary driver of resistance conferred by these *TAC1B* mutations.

BLOCK C

Transient Aneuploidy Drives Heteroresistance in *Candida albicans*

Eli Isael MACIEL[1], Cecile Gautier[1], Corinne Maufrais[1], Thomas Murphy[2], Chapman Beekman[2], Iuliana ENE[1]

[1]Pasteur, France. [2]Brown, USA

Abstract

Fungal infections are increasing globally and contribute substantially to morbidity and mortality. While antifungal resistance is the most recognized cause of treatment failure, subtler drug responses, such as heteroresistance, may also play a critical role. Heteroresistance refers to a phenomenon where a small subpopulation of cells within an otherwise susceptible strain transiently exhibits high-level resistance. here, we present the first detailed characterization of azole heteroresistance in *C. albicans*. We identify aneuploidy, as a frequent and reversible genetic alteration associated with the heteroresistant state. Upon removal of fluconazole, the aneuploid is often lost, coinciding with restored drug susceptibility. We further show that heteroresistance is linked to increased chromosomal instability, which can be induced genetically by disrupting transcription factors that regulate the cell cycle, or chemically by interfering with chromosome segregation. Importantly, we confirm the formation of heteroresistant cells in vivo, supporting their potential relevance in clinical settings.

BLOCK C

The Unique Efg1 Virulence Regulon in the Catheterized Bladder Environment

Alyssa La Bella[1], Nicholas Gervais[2], Rebecca Shapiro[2], Felipe Santiago-Tirado[1], Ana Flores-Mireles[1]

[1]University of Notre Dame, USA. [2]University of Guelph, Canada

Abstract

Urinary catheterization is a common procedure in hospitals and nursing home facilities. With an aging population, urinary catheterization will continue to be a necessary practice. However, these medical devices are one of the most common causes of hospital-acquired infections. Catheter associated urinary tract infections (CAUTIs) often lead to bloodstream infections, sepsis, and ultimately death. The fungal species *Candida albicans* has become the second most common causative agent of CAUTIs, and despite its frequent occurrence, relatively little is known about the pathogenesis of these infections and how to effectively treat them. Previously, we found that *C. albicans* CAUTIs are driven by the virulence factor Efg1 and its downstream adhesin, Als1. It is important to investigate critical factors for fungal CAUTIs, specifically those regulated by the transcription factor Efg1, to continue to unveil the mechanistic details of these infections. Here, for the first time, we identify the specific downstream factors that make up the Efg1 regulon in the unique urine microenvironment. Two of these Efg1 urine-regulated factors, *ECE1* and *EED1*, were found to be critical for bladder tissue colonization and neutrophil evasion during CAUTI. By identifying and characterizing the tissue-specific downstream targets of this essential transcription factor for *C. albicans* virulence, we can explore potential drug therapeutics that target these factors in hopes of finding antimicrobial-sparing strategies for these life-threatening hospital-acquired infections.

BLOCK C

Candida albicans TLO genes control genome instability and gene expression

Zinnat Shahina[1], Philip Hendricks[1,2], Andrew Woodruff[2], Abigail Ali[2], Matt Anderson[1,2]

[1]University of Wisconsin, USA. [2]The Ohio State University, USA

Abstract

The most expanded gene family in *Candida albicans* are a set of paralogs called the telomere-associated (*TLO*) genes. *TLOs* can be divided into three architectural groups (α, β, and γ) based on sequence variation. Importantly, the specific biological and molecular roles for each paralog is not well defined. Using CRISPR/Cas9, we constructed two *tlo* null lineages and used them to reintegrate single *TLO* genes into the *NEUT5L* locus. The *tlo*Δ/Δ strains could be easily distinguished from the wildtype; they displayed ruffled colony morphologies on YPD medium at 30°C that contained a mixture of hyphae, pseudohyphae, and yeast cells. Unexpectedly, the *tlo*Δ/Δ mutants grew robustly on canonically toxic sorbose and 2-deoxygalactose (2-DOG) media, whereas wildtype did not. Complementation of the *tlo*Δ/Δ mutant with α- and β-*TLO* genes restored susceptibility to both toxic carbon sources. Addition of *TLO*γ genes to *tlo*Δ/Δ mutants did not suppress growth on these media, with the exception of *TLOγ*4 that restored wildtype susceptibility to sorbose. Growth on these toxic sugars was partially reversible, suggesting that a regulatory mechanism by *TLO* genes governed survival. Simultaneously, loss of *TLO* genes increased genome instability as determined by *in vitro* evolution and altered telomere lengths. Taken together, we show that *TLOs* impact global phenotypes through a combination of promoting genome stability and direct gene regulation. This demonstrates that *TLO*s play a broad role in fundamental *C. albicans* physiology.

BLOCK C

Uncovering Factors Involved in Bacterial-Drug Synergy Against *Candida*

Allie Conner, Siham Hattab, Lindsey Stover, Robert Wheeler

University of Maine, USA

Abstract

The yeast, *Candida albicans*, and the bacterium, *Pseudomonas aeruginosa*, are opportunistic pathogens that co-colonize multiple sites within the human host, particularly in the immunocompromised lungs of Cystic Fibrosis (CF) and mechanically ventilated patients. CF patients co-colonized with both *C. albicans* and *P. aeruginosa* experience reduced lung function and poorer prognosis. We recently described how *P. aeruginosa* increases the effectiveness of the antifungal treatment Fluconazole (FLC) both *in vitro* and during co-infection in a vertebrate zebrafish infection model. We concluded that iron starvation by *P. aeruginosa* is one important component of this antagonistic interaction; however, it does not account for most of the effect. Here, we report that *Pseudomonas*-FLC synergy influences the fungal calcineurin pathway, a key fungal virulence factor linked to iron homeostasis. Specifically, it leads to a reduction in the translocation of transcription factor Crz1 to the nucleus. Reduced translocation of Crz1 diminishes the expression of calcineurin target genes related to fungal virulence. We also report preliminary results from screening a genome-wide nonredundant library of *P. aeruginosa* strain PA14 transposon insertion mutants to identify loss-of-synergy mutations. This unbiased approach has already implicated critical bacterial virulence factors, including the transcriptional regulator RhlR, which modulates quorum-sensing systems and the production of rhamnolipids. Identification of relevant bacterial pathways involved in *Pseudomonas*-FLC synergy will expand our knowledge of how bacteria communicate with *Candida* during co-colonization and infection, as well as provide insight into increasing the effectiveness of clinical treatments.

BLOCK C

An OMICS view of the interaction established between the commensal bacterium Lactobacillus gasseri and the pathogenic yeast Candida glabrata

Nuno P Mira[1,2], Nuno A Pedro[1,2], Lara Sousa[1,2], Margarida Duarte[1,2], Sandra C Pinto[1,2]

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

Abstract

The success of *C. glabrata* as a human commensal and pathogen depends on its ability to cope with a competing bacterial microflora that includes, in the vaginal and in the GI tract, the poorly characterized *L. gasseri* bacterial species. While in co-culture (both in planktonic or in biofilms) we found that *L. gasseri* reduces growth rate, viability and pathogenesis of *C. glabrata*, a phenotype that is, in part, prompted by the presence of acetate that enhances bacterial virulence towards the yeast. To better understand this result and foster the development of *L. gasseri*-based anti-Candida treatments, we explored a range of OMICS analyses that scrutinize, in detail, this bacteria-yeast interaction. In this work we will discuss those results, that allowed us to put together the landscape of how these two species interact, including the identification of metabolites produced by the bacterium that showed anti-*Candida* effect. Gathering results from phenOMICs experiments (undertaken in planktonic and in biofilms) we could also identify a set of *C. glabrata* mutants highly sensitive to the presence of *L. gasseri*. Leveraging these results, we chemically inhibited one of the signalling pathways determining tolerance of C. glabrata to L. gasseri resulting in a prominent loss of competitiveness of the yeast in a co-culture with the bacterium.

BLOCK C

Candida albicans exploits host immune activation for increased stress resistance

Candela Fernández Fernández[1], Sude Dinçer[1], Thomas Krüger[2], Olaf Kniemeyer[2], Axel A. Brakhage[2,3], Axel Dietschmann[1], Mark S. Gresnigt[1]

[1]Junior Research Group Adaptive Pathogenicity Strategies, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Germany. [2]Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Germany. [3]Institute of Microbiology, Friedrich Schiller University Jena, Germany

Abstract

Microbial success and persistence during infection requires appropriate adaptation to withstand the stresses in the host environment. *Candida albicans* coexistence on mucosal surfaces as part of the human mycobiome has fostered the evolution of adaptation strategies that allow the fungus to evade, escape, and counteract the host immune responses.

Using an *in vitro* inflammation-adaptation model, we have shown that *C. albicans* stress resistance is differentially increased when exposed to the culture supernatants of human immune cells. Particularly, supernatant of human macrophages stimulated with heat-killed *C. albicans* hyphae (but not yeast) increased its stress resistance. Proteomics on these supernatants revealed several proteins that might contribute to increase *C. albicans* stress tolerance.

We assessed how these proteins individually affect *C. albicans* growth potential under different immune-related stresses (oxidative and heat stress). Interestingly, we identified several proteins that differentially impact *C. albicans* stress resistance depending on the culture environment and the stress condition. Strikingly, disparate responses between different *C. albicans* strains were also observed, which might be linked to their origin (mucosal commensal vs. invasive isolate).

Host proteins related with iron metabolism and ROS detoxification, such as ferritin or glutathione reductase, could be exploited by the fungus for its growth under both oxidative and heat-stress conditions. This highlights the importance of scavenging iron and reduction equivalents from the host environment to overcome the immune-imposed stress.

In summary, we have shown that *C. albicans* can exploit factors released by human macrophages upon activation to overcome host-imposed stress.

BLOCK C

White-Brown switching controls phenotypic plasticity and virulence of *Candida auris*

Trinh Phan-Canh[1,2,3], Sabrina Jenull[1,2], Tamires Bitencourt[1,4], Saskia Seiser[3], Doris Moser[5], Marjan Motamedi[1,2,6], Anuradha Chowdhary[7], Adelheid Elbe-Bürger[3], Karl Kuchler[1,2]

[1]Max Perutz Labs Vienna, Vienna Biocenter Campus (VBC), Dr.-Bohr-Gasse 9, 1030, Vienna, Austria. [2]Medical University of Vienna, Center for Medical Biochemistry, Dr.-Bohr-Gasse 9/2, 1030, Vienna, Austria. [3]Medical University of Vienna, Department of Dermatology, Währinger Gürtel 18-20, 1090 Vienna, Austria. [4]Medical University of Vienna, Center for Medical Biochemistry, Dr.-Bohr-Gasse 9/2, 1030, Vienna, Vietnam. [5]Department of Oral and Maxillofacial Surgery, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria. [6]Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of. [7]Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India

Abstract

The skin-tropic human fungal pathogen *Candida auris* can cause life-threatening infections of high mortality in hospitals and healthcare settings. Clinical isolates from different clades display a pronounced heterogeneity in virulence traits such as antifungal susceptibility, stress adaptation and growth fitness. However, the mechanistic bases underlying intraspecies variations remain enigmatic. Here, we show that *C. auris* cell populations have multiple cell states that allow for reversible morphogenetic switching at high frequency resulting in White and Brown morphotypes. Further, we demonstrate that temperature and carbon sources are key drivers of morphogenesis. Importantly, White and Brown morphotypes show distinct phenotypes concerning stress tolerance, antifungal susceptibility and fitness on murine skin. Based on validated RNA-seq data, we show that W/B conversion engages both transcriptional activators including Wor1, Msn4, Crz2, Rca1 and the repressors Efg1. Heritable and reversible cell fate switching explains the phenotypic plasticity and variation in virulence traits of *C. auris*. The results set a foundation for exploring the therapeutic potential of regulators controlling morphogenesis in *C. auris*.

BLOCK C

Redefining Resistance Detection in *Candida*: A Transcriptomic Approach For Rapid AST and Heteroresistance Assessment

Elizabeth Yee[1], Brooks Burt[1], Eden Seyoum[1], Roby Bhattacharyya[1,2], Christina Cuomo[1,3]

[1]The Broad Institute, USA. [2]Massachusetts General Hospital, USA. [3]Brown University, USA

Abstract

Invasive fungal infections (IFIs) caused by *Candida* species represent a growing clinical challenge, exacerbated by rising antifungal resistance. Rapid and accurate antifungal susceptibility testing (AST) is critical for effective treatment, yet current methods are slow, and labor-intensive. To address this, we developed a simple and rapid transcriptional AST approach using NanoString Technologies' multiplexed RNA detection platform.

Building on our lab's success in using transcriptional signatures to distinguish bacterial resistance, we adapted this platform to characterize antifungal responses in clinically relevant *Candida* species: *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. auris*. Briefly, susceptible and resistant isolates of *Candida* species were cultured and treated with antifungals and samples collected over time for RNA-sequencing. Differential gene expression analysis was used to build custom NanoString probe panels to distinguish resistance for each *Candida* species treated with clinically relevant antifungals. Our panels for azole treatment of *C. albicans* and *C. parapsilosis* successfully discriminated susceptible from resistant isolates. Azole panels for *C. glabrata* and *C. auris* performed moderately well, likely due to intrinsic resistance. An echinocandin panel was tested for *C. glabrata* where azole resistance is prevalent. Interestingly, several resistant isolates exhibited unexpected responses to micafungin. Population analysis profiling confirmed these were due to heteroresistance— and confirmed subpopulations vary in drug susceptibility. These findings establish transcriptional profiling as a promising tool for rapid *Candida* AST and highlight the need to further explore heteroresistance measurements to improve diagnostics for drug-resistant infections.

BLOCK C

Unmasking the Fungal Factor: *Candida albicans* Dysbiosis and Gastrointestinal Inflammation in Cystic Fibrosis

Daniel Gutzmann, Ana Victoria de Sousa Bezerra, Sarvesh Surve, Jay Goddard, Devon Gokey, Deborah Hogan

Dartmouth College, USA

Abstract

While cystic fibrosis transmembrane conductance regulator modulators have transformed cystic fibrosis treatment, gastrointestinal symptoms and complications remain prevalent for persons with CF (pwCF). The gut microbiome, comprising bacterial and fungal components, is pivotal in modulating gastrointestinal health. Although the bacterial microbiome has been extensively studied, the role of intestinal fungi, particularly *Candida albicans*, remains relatively unexplored. To address this gap, we analyzed colonoscopy aspirates from the Dartmouth CF Research Center (DartCF) specimen bank and discovered stark differences in yeast abundance and diversity between pwCF and healthy volunteers. pwCF exhibited a higher yeast abundance and a striking loss of mycobiome heterogeneity, with most individuals being monocolonized by either *C. albicans* or *Candida lusitaniae*. This dominance of *C. albicans* starkly contrasts with the relative diversity of the bacterial microbiome. Furthermore, our findings suggest pathogen adaptation by *C. albicans* in the CF gut environment, with some isolates harboring loss-of-function (LOF) mutations in the *NRG1* gene, a key transcriptional repressor involved in filamentation and metabolic adaptation. Our research will analyze differences in the bacterial microbiome of pwCF to investigate the interactions between *C. albicans* and the bacterial community, aiming to understand the factors driving *C. albicans* colonization, the emergence of Nrg1 LOF mutations, and the implications of this dominance and adaptation for gastrointestinal health in pwCF.By elucidating these mechanisms, we aim to identify potential therapeutic approaches to mitigate gastrointestinal symptoms and improve the quality of life for pwCF and learn more about the niche *Candida* spp. occupy in the microbiome.

BLOCK C

Nutrient availability and growth conditions shape long-term viability in *Candida albicans*

Marina Druseikis, Natali Matry, Judith Berman

Shmunis School of Biomedical and Cancer research, Tel Aviv University, Israel

Abstract

Nutrient availability drives cell growth and division; conversely, nutrient depletion induces cellular aging and death, or entry into a temporary and reversible non-dividing state called quiescence. We tested the effect of gradual depletion of specific macronutrients (e.g. carbon, nitrogen, or phosphate) and measured culture growth dynamics, cell morphology, mitochondrial morphology, and long-term viability (i.e. entry into and subsequent exit from quiescence) in *Candida albicans*. We found that long-term cultivation (> 4 days) in rich media (YPD) causes a rapid decline in the viability of the standard lab strain (SC5314), but does not reduce viability in multiple clinical isolates. Additionally, variations in common growth parameters, such as flasks vs tubes or shaking with or without glass beads, greatly affect the long-term viability of some C. albicans isolates. Taken together, the results show that heterogeneity in cell size, morphology, and the entry into and exit out of quiescence are a feature, not a bug, of aged *C. albicans* cells. Furthermore, the ability to enter and exit the quiescent state may contribute to the degree of drug susceptibility seen among the diversity of C. albicans isolates.

BLOCK C

Disclosing azole resistance mechanisms in an azole-resistant *Candida glabrata* clinical strain that encodes a wild-type CgPDR1 allele

Maria Joana Pinheiro[1,2], Marta Pedroso[1,2], Josie Parker[3], Fábio Fernandes[1,2], Nuno Pereira Mira[1,2]

[1]iBB, Institute for Bioengineering and Biosciences, Instituto Superior Técnico—Department of Bioengineering, Universidade de Lisboa, Lisboa 1049-001, Portugal, Portugal. [2]Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Lisboa 1049-001, Portugal, Portugal. [3]Molecular Biosciences Division, School of Biosciences, Cardiff University, Cardiff, Wales, United Kingdom, United Kingdom

Abstract

Candida glabrata rapidly acquires resistance to azoles, reducing the efficacy of treatments and increasing mortality/morbidity of infected patients. A lot of knowledge has been gathered on the mechanisms by which *C. glabrata* acquires resistance to azoles in lab strains, but much less is known in clinical strains. Our laboratory has been investigating this issue and came across one azole-resistant *C. glabrata* strain - ISTB218 – whose phenotype could not be attributed to a CgPdr1 gain-of-function allele, the most common resistance mechanism reported until so far in clinical strains. Transcriptomic and genomic analyses of ISTB218 pointed to several other mediators of the resistance phenotype, in particular, genes involved in synthesis of ergosterol (other than ERGs). Follow-up on those results led us to identify one enzyme, up-regulated in ISTB218 cells, that serves as an alternative to Erg11 bypassing the azole-induced inhibition of this enzyme. Consistently, fluorescence microscopy imaging of ISTB218 cells clearly shows a prominent accumulation of ergosterol in vesicles (a trait not observed in susceptible strains nor in strains encoding CgPdr1 GOF mutants), likely serving as reservoirs when under azole stress. Lipid analysis of whole-ISTB218 cells confirm this hypothesis. The results presented in this work pave the way for the understanding of the acquisition of resistance to azoles *in vivo* beyond the mechanisms that involve CgPdr1 hyper-active alleles and thus highlighting other relevant players, a knowledge essential to overcome resistance and promote more efficient diagnostic tools.

BLOCK C

Antifungal lead development targeting Cdc14 phosphatase

Kevin Velazquez-Marrero, Emily Danzeisen, Ahmed AbdelKhalek, Jinmin Miao, Jiajun Dong, Zhong-Yin Zhang, Mark Hall

Purdue University, USA

Abstract

There are few effective antifungal drugs for treating invasive fungal infections. Increasing pathogen resistance to available drugs necessitates the identification of novel targets and therapeutics with unique modes of action. Our group recently demonstrated that Cdc14 phosphatase, which is invariant in Dikarya fungi, is required for virulence of *Candida albicans* in mouse models of invasive candidiasis. Cdc14 is also important for virulence in several phytopathogens, suggesting a conserved function important for host infection. Cdc14 has a unique active site structure and substrate specificity that we have argued should be amenable to selective inhibitor development. Here, we report a novel competitive inhibitor scaffold targeting the Cdc14 phosphatase family based on a phosphotyrosine mimetic, and demonstrate its potential as an antifungal lead. Our inhibitor exhibits broad activity against Cdc14 enzymes from diverse fungal pathogens and minimal activity against other related phosphatases. It effectively suppresses hyphal growth of *C. albicans* in embedded agar and in a mouse model of invasive candidiasis, phenotypes associated with Cdc14 loss of function mutants. Even without optimization, this initial inhibitor compound significantly extended mouse survival after invasive infection and exhibited no toxicity. Ongoing efforts for further optimizing and characterizing this scaffold, and generating additional Cdc14-selective inhibitors, will be presented and discussed.

BLOCK C

Functional characterisation of sugar kinases in *Candida glabrata* and their contribution to virulence

Jolien Vreys, Julie Van Olmen, Eline Schraepen, Stefanie Wijnants, Patrick Van Dijck

Laboratory of Molecular Cell Biology, KU Leuven, Belgium

Abstract

Candida glabrata is a commensal fungus in the human body but can become pathogenic, leading to potentially fatal infections. While the significance of several virulence factors has been described, an increasing number of studies highlight the importance of central carbon metabolism in fungal pathogenicity. In this study, we investigated sugar metabolism in *C. glabrata*, focusing on sugar kinases that catalyse sugar phosphorylation during the first step of glycolysis. Based on homology with sugar kinases identified in other fungi, we found five putative sugar kinases in *C. glabrata*. Enzymatic assays revealed that only three are catalytically active: two hexokinases (Hxk2 and Hxk2b) and one glucokinase (Glk1), each with different substrate specificities and affinities. We demonstrated that trehalose-6-phosphate inhibits hexokinase activity but not glucokinase activity, consistent with findings in other fungi. Deletion of all three sugar kinases resulted in an inability to grow on fermentable carbon sources, while only minor growth differences were observed when the deletion strains were grown on non-fermentable carbon sources. Interestingly, *GLK1* deletion resulted in decreased glucose uptake, whereas deletion of the hexokinases had the opposite effect. Loss of sugar kinases impaired multiple virulence traits, including adhesion, biofilm formation, and stress tolerance. Furthermore, *C. glabrata* killing by macrophages was enhanced in double and triple sugar kinase deletion strains. Nevertheless, phagocytosis efficiency remained unaffected. Finally, we showed that in a murine vaginal infection model, sugar kinase deletion rendered *C. glabrata* avirulent. These findings emphasise the importance of sugar kinases not only in energy production but also in virulence.

BLOCK C

Candida albicans Erg251 selectively governs sterol C4-methyl oxidase activity under extreme hypoxia and supports fungal pathogenesis

Bonnie Yiu[1], Zhongle Liu[1], Pauline Basso[2], Xuefei Chen[3], Disha Patel[4], Taylor Davie[1], Saif Hossain[1], Ci Fu[1], Linda S. Archambault[1], Dustin Duncan[4], Gerard D. Wright[3], Suzanne M. Noble[2,5], Nicole Robbins[1], Leah E. Cowen[1]

[1]Department of Molecular Genetics, University of Toronto, Canada. [2]Department of Microbiology and Immunology, University of California San Francisco, USA. [3]David Braley Centre for Antibiotics Discovery, M.G. DeGroote Institute for Infectious Disease Research, Department of Biochemistry and Biomedical Sciences, McMaster University, Canada. [4]Department of Chemistry, Brock University, Canada. [5]Department of Medicine, Division of Infectious Diseases, University of California San Francisco, USA

Abstract

Intestinal-colonizing *Candida albicans* is a primary source of systemic infection where it can translocate across the intestinal barrier into the bloodstream leading to disseminated candidiasis with mortality rates approaching 50%. The growing threat of antifungal resistance coupled with a limited therapeutic arsenal highlights the urgent need to deepen our understanding of fungal biology and identify novel antifungal targets. To persist in the gastrointestinal tract, *C. albicans* must adapt to complex environmental conditions, including extreme hypoxia. In this study, we performed a functional genomic screen to identify genes important for *C. albicans* fitness under extreme hypoxic conditions (EHC). We identified *ERG251*, one of two *C. albicans* sterol C4-methyl oxidase genes in the ergosterol biosynthesis pathway, as specifically required for fitness under EHC. We demonstrated that the loss of *ERG251* impairs ergosterol production and severely compromises fungal growth in low oxygen conditions. Furthermore, we established that *ERG251* is critical for *C. albicans* virulence and colonization in mouse models of systemic infection and commensalism, respectively. Through random mutagenesis, we identified key amino acid residues important for the enzymatic function of Erg251 under extreme hypoxia. Finally, we leveraged pharmacological inhibitors of sterol C4-methyl oxidases to highlight the potential of targeting *ERG251* as a therapeutic strategy *in vitro* and in a nematode infection model of *C. albicans* infection. Overall, our work provides a comprehensive analysis of hypoxic adaptation in *C. albicans* and highlights the critical role of *ERG251* in fitness under EHC, contributing to the expansion of potential antifungal targets.

BLOCK C

Spatial Control of Nrg1 Degradation Defines Cell Fate in *Candida albicans* Hyphae

Soledad Píriz-Antúnez[1], Manuel Humánez-Marín[1], Encarnación Dueñas[2], Antonio Esperilla-Muñoz[1], Nuria Trevijano-Contador[1], Carlos R Vázquez de Aldana[2], Jaime Correa-Bordes[1]

[1]Dpto. Ciencias Biomédicas. Universidad de Extremadura, Spain. [2]Instituto de Biología Funcional y Genómica. USAL-CSIC, Spain

Abstract

Candida albicans exhibits remarkable phenotypic plasticity, enabling reversible transitions between yeast and hypha forms. In hyphae, only the apical cell grows and divides, while subapical cells remain arrested, possibly due to asymmetric segregation of cellular components. Hyphal development requires transient degradation of the Nrg1 repressor, which controls virulence-related hypha-associated genes (HAGs). Although several genes regulating Nrg1 degradation are known, its spatial control within mature hyphae was unclear. Using fluorescence microscopy and FRAP in several conditional mutants, we demonstrate that Nrg1 degradation occurs specifically in the apical cell and depends on the Ndr kinase Cbk1 and the exportin Crm1. This spatial regulation ensures HAG expression in the apical cell while repressing it in subapical cells. Disruption of this mechanism, by inactivation of Cbk1 or Crm1, leads to Nrg1 nuclear accumulation and loss of HAG expression in the tip cell. Possible mechanisms linking Cbk1 to Nrg1 degradation will be discussed.

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Poster

BLOCK A

Discovery and Characterization of Novel Small-Molecule Antifungal Drugs Proposed by Machine Learning Models

Eileen Bates[1], Autumn Arnold[2], Iwona Skulska[1], Jonathan Stokes[2], Rebecca Shapiro[1]

[1]University of Guelph, Canada. [2]McMaster University, Canada

Abstract

Fungal infections are notoriously difficult to treat, causing an estimated 3.8 million deaths annually. Only four classes of antifungal drugs have been approved, and resistance to these molecules represents a growing threat. Development of novel antifungals is challenging because traditional chemical screens are often not optimized for fungal targets and cannot sufficiently probe antifungal-amenable chemical space. Recently, machine learning models have been successfully applied to address similar challenges in antibiotic discovery, but the application of these models towards fungal pathogens remains extremely limited.

We aim to leverage contemporary machine learning approaches to discover novel small-molecule antifungal drugs against *Candida albicans*. We screened a library of over 11,000 bioactive compounds for their ability to inhibit *C. albicans* growth, then used these datasets to train molecular property prediction models. These were applied to perform predictions on approximately 12 million molecules from the ZINC15 database of commercially available small molecules. Based on these predictions, we identified 2 structurally novel compounds with potent antifungal activity against *Candida albicans*, as well as other diverse fungal pathogens and antifungal-resistant clinical isolates. Both compounds have promising toxicity profiles in human embryonic kidney (HEK293) and *Caenorhabditis elegans* models. Currently, we are further profiling the activity of both compounds and investigating their mechanisms of action. This work represents a powerful new approach to antifungal drug development, which is crucial for decreasing the burden of fungal pathogens on human health.

BLOCK A

Determining the individual function and effect of structural variation among the *TLO* gene family members of *Candida albicans*

Philip Hendricks[1], Anirudh Ventkataraman[1], Zinnat Shahina[1], Shahed Shazib[2,3], Abigail Ali[4,3], Matthew Anderson[1,3]

[1]University of Wisconsin-Madison, USA. [2]Purdue University, USA. [3]Ohio State University, USA. [4]University of Richmond, USA

Abstract

The clinical relevance of *Candida* species coincides with the expansion of several gene families involved in pathogenesis, such as adhesins and aspartyl-proteases. Among the *Candida* species *C. albicans* is the most clinically important and has experienced an even greater expansion of another gene family, the telomere-associated (*TLO*) genes. *TLOs* are present as 14 paralogs broken into three architectural groups (α, β, and γ) in the genome reference strain and are functional homologs of the Med2 subunit of the yeast Mediator transcriptional regulatory complex. However, their individual molecular and biological roles in pathogenesis and adaptation remain unknown. To elucidate their individual roles, a complete *TLO* knockout was constructed in the genome reference strain using CRISPR-Cas9 mutagenesis. This *tlo* null mutant served as the platform to build a panel of single *TLO* addback strains. The *tlo* null strain showed radical changes in morphological state from wildtype in standard conditions that could be complemented by addition of any *TLO*α or *TLO*β gene but not most *TLO*γ genes. Similarly, other simple phenotypes could be predicted by the architectural group of the *TLO* re-integrated into the genome. Interestingly, addition of a truncated *TLO*γ gene, *TLO*γ4, restored wildtype phenotypes, consistent with the *TLO*α and not *TLO*γ genes. We are currently performing transcriptional and phenotypic analysis of single *TLO* genes and their encoded domains to determine how individual paralog function evolved and if each gene can provide unique responses that can contribute to fitness.

BLOCK A

Conservation of function between *Candida albicans* filamentation regulators and *Candida auris* genes.

Makenna May, Carson Noyes, Ian Cleary, Derek Thomas

Grand Valley State University, USA

Abstract

Candida auris has been declared an organism of concern due to its apparent ability to spread between patients *via* fomites. It is inherently drug resistant and a better understanding of this pathogen is urgently needed. In *Candida albicans*, pathways that regulate morphology can also regulate drug resistance so we have been studying similar regulatory mechanisms in both organisms. In particular, we have been looking at a family of phosphatases and their targets. These phosphatases share high sequence similarity with each other and between the two species, although *C. albicans* and *C. auris* have a different complement of genes. We found that over-expressing either the *C. albicans* versions or the *C. auris* versions in *C. albicans* was able to impair hyphal growth in some conditions. This is surprising given that *C. auris* does not form true hyphae. It is not yet clear what function these genes have in *C. auris* although over-expression in *C. albicans* resulted in slightly increased sensitivity to caspofungin. We have continued this work by expressing co-regulated genes from *C. albicans* in *C. auris* to see if there is conservation of the function in *C. auris* as well as correlation with phosphatase function.

BLOCK A

Development and delivery of novel anti-fungal agents targeting Candida species

Dev Arya

Clemson University, USA

Abstract

Candida species are the leading source of morbidity and mortality worldwide that are caused by pathogenic fungal infections. Increasing antifungal resistance dictates that a new way of fighting these multidrug resistant fungi must be utilized. Development of new approaches (small and large molecules) and their uptake in Candida species, especially in biofilms, presents some unique problems. We have investigated the development of novel motifs to facilitate uptake of such molecules in Candida species, to bypass some of the antibiotic resistance mechanisms. Such new approaches in antifungal drug development will be shared in this presentation.

BLOCK A

Screening of the ChemDiv Antifungal Library Reveals Novel Inhibitors of Biofilm Formation in *Candida albicans* and *Candida auris*

Jesus Vazquez Rodriguez[1], Jose Lopez-Ribot[1], Fabian Moreno-Alvitia[2]

[1]University of Texas at San Antonio, USA. [2]Centro Nacional de Biotecnologia, Spain

Abstract

Candida albicans and *Candida auris* are opportunistic pathogenic fungi classified as critical-priority pathogens by the World Health Organization due to their high clinical prevalence, ability to form biofilms, and increasing resistance to antifungal agents. In this study, we conducted a large-scale screening of 10,000 compounds from the ChemDiv Antifungal Library using the XTT 96-well microtiter plate model of biofilm formation to identify novel inhibitors of biofilm formation in *C. albicans* and *C. auris*. The initial screening identified 39 and 18 hits for *C. albicans* and *C. auris*, respectively, based on predefined thresholds for biofilm inhibitory activity (> 80% for *C. albicans* and > 70% for *C. auris*). After dose-response confirmatory assays, we selected eight leading compounds which exhibited potent and selective activity, with IC_{50} values below 15 µM for *C. albicans* and below 28 µM for *C. auris*. Several of these leading compounds also demonstrated inhibitory activity against preformed biofilms and planktonic cells in a dose-dependent manner. Structural analysis revealed diverse chemical scaffolds, including benzimidazole, salicylanilide, and aminoacridine, with known or predicted bioactivity. Importantly, four leading compounds exhibited dual-species activity, underscoring their potential as broad-spectrum antifungal candidates. These findings contribute to the antifungal drug discovery field by identifying chemical scaffolds with potential biofilm-targeting properties against *Candida* spp. and may provide preliminary leads for developing future antifungal agents.

BLOCK A

ECE1 in closely related non-*albicans Candida* species

Tim B. Schille[1,2], Hans May[1], Stefanie Allert[1], Jakob L. Sprague[1], Toni Gabaldón[3], Bernhard Hube[1,2]

[1]1. Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knoell-Institute, Jena, Germany. [2]2. Cluster of Excellence Balance of the Microverse, Friedrich Schiller University Jena, Germany. [3]4. Comparative Genomics group, Institute for Research in Biomedicine and Barcelona Supercomputing Centre, Spain

Abstract

Hypha formation of *Candida albicans* is a main pathogenic trait, associated with strong induction of the *ECE1* gene. The Ece1 polyprotein is processed into smaller peptides, including candidalysin – a pore-forming peptide toxin, well characterized as key factor for hypha-associated host cell damage, but also found to support gut colonization. While orthologous *ECE1* genes were identified in the closely related pathogenic species *C. dubliniensis* and *C. tropicalis*, we also found *ECE1* genes in *C. viswanathii* and several related non-pathogenic species including *C. maltosa* and *C. sojae*.

In this study we aimed to characterize the potential role of *ECE1* genes in these non-*albicans Candida* species with regard to their environment. Using *in silico* analysis of *ECE1* sequences, we found a strong conservation, including Ece1 processing sites and predicted protein structure. These findings suggest that this protein evolved from a common ancestor of these species and was retained as an important fitness factor.

Non-*albicans Candida* species were phenotypically characterized, revealing differences for fungal growth and stress sensitivity. None of the species matched *C. albicans*' cytotoxicity during *in vitro* infection of human cells. However, synthetic non-*albicans* candidalysins clearly showed cytotoxicity against human as well as bacterial cells. Fluorescent transcriptional reporter strains were produced to characterize expression conditions for *ECE1* in non-*albicans* species to further shed light on the functional role of Ece1 for each species.

Our observations suggest a multi-functional role of candidalysin variants beyond *C. albicans* host infections and highlight the evolution within other *Candida* species.

BLOCK A

A novel strategy to combat human fungal pathogen *Candida glabrata*

Debarati Mondal, Shubhashis Das, Mohd Waseem, Jitendra Kumar Thakur

International Centre for Genetic Engineering and Biotechnology (ICGEB), India

Abstract

The global burden of fungal infections has become a significant concern contributing 1.5 million deaths annually. Among these fungal pathogens, *Candida* species have emerged one of the most common causes of mucosal, nosocomial, and systemic infections. Over the time, the emergence of immune-suppressive population and drug-resistant strains led to the urgent need of novel antifungals against such invasive life-threatening fungal diseases. Here in this study, we investigated the mode of action and therapeutic potential of two ellagitannin compounds against *Candida glabrata.* Our data shows that these compounds cause mitochondrial perturbation in fungal system and affect the intracellular iron homeostasis pathway serving as a novel mechanism from currently approved drugs. However, both of these compounds were extracted and purified from *Terminalia chebula* and found that it has minimal cytotoxic effect in human cell lines. The synergistic combination of these compounds with current available drugs prevented the infection of *Candida glabrata* and significantly improved fungal clearance by mice peritoneal macrophages. Additionally, immunophenotyping analysis showed that these ellagitannin compounds can enhance a potent immune response in mice cells. Collectively, this work signifies the inhibitory and immunomodulatory potential of these bioactive compounds which could be used as novel antifungal formulations as well as in the concurrent antifungal therapy against *Candida* spp.

BLOCK A

Differential modulation of complosome by colonizing and VVC-associated *Candida (C) albicans* strains in vaginal epithelial cells

Samyr Kenno[1], Eva Pericolini[1], Robert T. Wheeler[2,3], Wilfried Posch[4], Andrea Ardizzoni[1], Manola Comar[5], Natalia Pedretti[5], Luca Spaggiari[6], Samuele Peppoloni[1]

[1]Department of Surgical, Medical, Dental and Morphological Sciences with Interest in Transplant, Oncological and Regenerative Medicine, University of Modena and Reggio Emilia, Modena, Italy. [2]Department of Molecular and Biomedical Sciences, University of Maine, Orono, Maine, USA. [3]Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, Maine, USA. [4]Institute of Hygiene and Medical Microbiology, Medizinische Universität Innsbruck, Innsbruck, Austria. [5]Department of Clinical, Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy. [6]Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia Modena, Italy

Abstract

Introduction

C. albicans is a common component of the vaginal microbiota but can also cause vulvovaginal candidiasis (VVC). Intracellular complement system, also known as complosome, contributes to local immune responses, however its involvement in VVC remains elusive.

Methods

An *in vitro* infection model using human vaginal epithelial cells (VECs) was employed to compare a colonizing *C. albicans* strain from a healthy woman with a pathogenic strain from a VVC patient. Flow cytometry assessed the intracellular C3, C5, their products C3a, C3b, C5a, and receptors C5aR1 and C3aR. ELISA quantified C3a and C5a release. RNA-seq analyzed transcriptomic changes and a functional assay evaluated Cathepsin D activity in VECs.

Results

Neither strain alter C3 levels but both increased C3a and C3b compared to uninfected VECs; however, VVC strain induced lower C3b production. While colonizing strain did not affect intracellular C5 or C5a, VVC strain caused marked reduction of both. Only colonizing strain substantially increased intracellular C5aR1. In contrast, C3aR similarly increased in VECs infected with both strains compared to those uninfected. A greater C3a release was induced by colonizing strain, with no C5a increase after infection with both strains. Expression of Cathepsin genes and Cathepsin D activity were reduced in VECs infected with VVC strain compared to those infected with colonizing strain.

Conclusion

The VVC strain compromises C5a/C5aR1 axis and reduces C3a/C3aR axis through the regulation of Cathepsins. Our study provides the first evidence linking the complosome to VVC pathogenesis, highlighting the C5a/C5aR1 axis as a potential therapeutic target in fungal infections.

BLOCK A

Treatment of Candida albicans with decreasing subinhibitory concentrations of caspofungin induces a stepwise increase in β-(1,3)-glucan unmasking

Kaley Taylor, Millen Tesfamariam, Todd Reynolds

University of Tennessee-Knoxville, USA

Abstract

Candida albicans is an opportunistic fungal pathogen that can evade immune detection by masking β-(1,3)-glucan (a crucial cell wall polymer) with a layer of glycosylated proteins. β-(1,3)-glucan is essential for cell wall integrity but is also a major pathogen-associated molecular pattern recognized by innate immune receptors. The echinocandin caspofungin inhibits β-(1,3)-glucan synthesis and induces β-(1,3)-glucan unmasking at sites of cell wall growth, thereby enhancing immune recognition. Nevertheless, the signaling cascades that drive caspofungin-induced unmasking remain poorly understood. Surprisingly, there is an inverse correlation between caspofungin concentration and the level of unmasking. When treated with decreasing subinhibitory concentrations of caspofungin (10-2.5 ng/mL) for 30 minutes, both mother and daughter cells show increased unmasking. However, treatment with a higher concentration approaching ⅜ the minimum inhibitory concentration (46.9 ng/mL) for the same time results in reduced unmasking primarily confined to the budding daughter cell. This concentration-dependent shift in unmasking suggests distinct signaling pathways or regulatory mechanisms may be engaged at different caspofungin concentrations. To better understand these processes, we are performing RNA sequencing at subinhibitory concentrations of caspofungin to capture differential gene expression associated with this gradient of unmasking phenotypes. This will help identify key transcriptional changes and potential regulators driving the response. Additionally, we will use shotgun proteomics of cell wall-associated proteins enriched via tyramide signal amplification and click chemistry to uncover cell surface proteins involved in unmasking. Together, these approaches will help identify molecular players and signaling networks involved in caspofungin-induced unmasking.

BLOCK A

Coupling auxin-inducible protein degradation with animal infection models as a tool for antifungal target discovery.

Emily Danzeisen, Michelle Lihon, Ahmed AbdelKhalek, Mark Hall

Purdue University, USA

Abstract

There is an urgent need for new antifungal therapies and targets. To address this need, we are developing the auxin-inducible degradation (AID) system in *Candida* species for use in animal infection models. The AID system enables rapid degradation of a target protein upon addition of the plant hormone auxin and has been used in various eukaryotic organisms for protein characterization. We hypothesize that AID can be applied in the realm of drug discovery as a platform to screen for potential antifungal targets, with auxin acting as a universal drug surrogate. We predicted that auxin would induce degradation of a target protein *in vivo* during infections, reducing fungal burden and improving survival when the target is important for virulence. To validate this approach, we used two well-established animal models of invasive candidiasis – *Galleria mellonella* larvae and mice – and tested the known *C. albicans* virulence protein Cdc14 and the essential protein Glc7. During infection, auxin treatments significantly increased survival of the host and reduced fungal burden, indicating decreased pathogen virulence and effective *in vivo* degradation of the protein targets. This novel application of the AID system should enable high-throughput screening of pooled strain libraries to identify potential antifungal targets. We predict this approach will also be applicable in other fungal pathogen species.

BLOCK A

Intracellular iron homeostasis is a key factor regulating azole cidality, tolerance and susceptibility in Candida albicans

Wouter Van Genechten, Patrick Van Dijck

KU Leuven, laboratory for Molecular Cell Biology, Belgium

Abstract

Fungal infections are combatted using three main classes of antifungals, of which the most widespread treatment are the azoles which are classically considered to be fungistatic. Slow growth of *Candida albicans* at supra-MIC concentrations of fluconazole, termed tolerance, is routinely observed. A combination therapy resulting in the eradication of this fungistatic and tolerant character would be a valid therapeutic strategy and indeed, we discovered that a combination of the antibiotic doxycycline and fluconazole (FLC) has such an effect. We hypothesized that iron and mitochondrial function may be the target of the synergistic combination. A proteomics analysis of purified mitochondria obtained from FLC + Fe treated cells hinted that iron alleviated the FLC stress and that intracellular iron homeostasis, more specifically the vacuolar iron exporter Smf3, might be a key factor during FLC treatment. Whilst doxycyline and fluconazole display a synergistic profile to the wild type, deletion of SMF3 removed this synergy. This suggests that deletion of SMF3 mimicks the doxycycline effect. A ROS assay revealed that in the SMF3 deletion, FLC treatment results in a strong ROS accumulation similar to that displayed by the FLC + DOX treatment in the WT. Furthermore, overexpressing the mitochondrial super oxide dismutase SOD2, lowers the displayed ROS accumulation and restored the synergy between DOX and FLC in the smf3Δ/Δ background. Interestingly, even though synergy was significantly altered in this strain, no effect on either cidality or tolerance was observed. Therefore, we were able to partially dissect the susceptibility profile of C. albicans to FLC.

BLOCK A

Unravelling the infection dynamics of *C. albicans* clinical isolates in gut epithelial cells using fluorescent reporter cell lines

Myrto Katsipoulaki[1], Taniya Banerjee[1], Marisa Valentine[1], Verena Truemper[1], Bernhard Hube[1,2], Sascha Brunke[1]

[1]Department of Microbial Pathogenicity Mechanisms, Hans Knoell Institute, Jena, Germany. [2]Institute of Microbiology, Friedrich Schiller University, Jena, Germany

Abstract

Despite its commensal nature, *Candida albicans* can cause severe disease in predisposed humans. During its transition from commensal to pathogen within the gut, one of the first host cells it interacts with are intestinal epithelial cells. The result of these interactions between the host and the fungus is host cell death. Even though, some studies have explored which type of cell death is activated, information on induction of programmed cell death pathways is still missing. Additionally, the vast majority of studies so far have been based on SC5314, a clinical isolate commonly described as more virulent than most others.

To understand the dynamics of host cell death, and the interactions between gut epithelial cells and various *C. albicans* clinical isolates, we have here employed a new set of fluorescent reporter lines for host cell death and stress. Specifically, we have created and used a fluorescent reporter for redox status, based on a roGFP2 fluorophore, a switch-on reporter for live detection of apoptosis, and an ASC speck formation reporter for inflammasome activation.

With these reporters, we have found 12 clinical isolates that cause damage at levels similar to SC5314, but show differences in the dynamics of the infection. For example, isolate C227 causes higher levels of apoptosis at 48 hpi, while the adhesion and invasion levels remain lower than during infection with SC5314. With our expanding set of reporter cells, we will soon be able to fully dissect and understand the infections dynamics between different *C. albicans* isolates and host cells.

BLOCK A

Expanding the network of *Candida albicans* chlamydospore regulation

Lasse van Wijlick, Iryna Denega, Nelson Mounguengue, Christophe d'Enfert, Sophie Bachellier-Bassi

Institut Pasteur, France

Abstract

The human opportunistic pathogen *C. albicans* can adopt various morphological forms depending on its environment. Most of them have been shown to provide adaptative functions (mating/opaque; dissemination/yeast; invasion/hyphae), but those of chlamydospores remain elusive. These large cells, usually borne by pseudohyphae, can be efficiently produced in vitro in rather harsh conditions (nutrient-poor medium, low temperature, microaerophilia) in strain-dependent conditions but have never been conclusively linked to pathogenesis.

We have previously shown that Rme1 is the central regulator of chlamydospore formation, which is also controlled by transcriptional regulators of hyphal growth (Efg1, Ndt80, Nrg1, and Sfl1), by the stress-activated protein kinase Hog1, as well as by the TOR and Ras-cAMP-PKA signalling cascades. How these signalling pathways operate to form chlamydospores remains unclear.

Here we used transcriptomic time-course analysis of a strain grown in yeast- or chlamydospore-inducing conditions, followed by mutant analyses, to identify novel positive and negative regulators of chlamydospore formation. We then compared the transcriptomics data obtained upon overexpression of these regulators to identify a set of co-regulated genes. This led to the expansion of the transcriptional network governing chlamydospore formation.

BLOCK A

Targeting epigenetic regulators to overcome drug resistance in the emerging human fungal pathogen *Candida auris*

Changbin Chen

Shanghai Institute of Immunity and Infection, China

Abstract

The frequent use of antifungal agents has contributed to the emergence of previously rare or unidentified drug-resistant fungal species, such as *Candida auris*, which presents mortality rates exceeding 40% and antifungal resistance rates surpassing 90%. The rise of life-threatening infections caused by drug-resistant fungal pathogens, coupled with the limited arsenal of effective antifungal agents, necessitates the urgent development of novel strategies to combat multidrug resistance. In this study, we systematically evaluated the role of post-translational modifications (PTMs) of histone H3 in drug resistance in *C. auris*, focusing on acetylation mediated by the acetyltransferases Gcn5 and Rtt109, as well as methylation by the methyltransferases Notably, we discovered that *GCN5* depletion and the subsequent loss of histone H3 acetylation downregulate key genes involved in ergosterol biosynthesis and drug efflux, resulting in increased susceptibility to major antifungal classes. Gcn5 regulates cell wall integrity and echinocandin resistance through modulation of the calcineurin signaling and the transcription factor Cas5. In invasive infection models, the deletion of *GCN5* significantly reduced the virulence of *C. auris*. Furthermore, we demonstrated that the Gcn5 inhibitor $CPTH_2$, when combined with caspofungin (CAS), exhibits a synergistic effect against *C. auris* in both *in vitro*and *in vivo* models without significant toxicity to human cells or mice. In conclusion, these findings highlight the critical role of Gcn5 in the resistance and pathogenicity of *C. auris*, positioning it as a promising therapeutic target for combating invasive fungal infections.

BLOCK A

Deciphering the target of a novel antifungal compound via chemogenomics, metabolomics, and molecular modeling

Jana Nysten[1], Wout Van Eynde[2], Yunjin Lee[3], Eliane Vanhoffelen[4], Tine Van Win[4], Greetje Vandevelde[4], Arnout Voet[2], Nicole Robbins[3], Leah Cowen[3], Patrick Van Dijck[1]

[1]Laboratory of Molecular Cell Biology, KU Leuven, Belgium. [2]Laboratory of Biomolecular Modelling and Design, KU Leuven, Belgium. [3]Cowen lab, University of Toronto, Canada. [4]Biomedical MRI Unit/MoSAIC, KU Leuven, Belgium

Abstract

The global rise in fungal infections, driven by expanding at-risk populations and growing antifungal resistance, highlights the urgent need for new therapies with novel mechanisms of action. Existing antifungal drugs are limited to a few classes, and their efficacy is increasingly compromised by resistant pathogens.

To address this challenge, we screened 20,000 drug-like molecules and identified Z247611722, a novel compound with potent fungicidal activity against multiple *Candida* species, including a clinically relevant fluconazole-resistant isolate. Its synergistic interaction with fluconazole further highlights its promise for combinatorial therapy.

Chemogenomic, metabolomic, and phenotypic analyses revealed that Z247611722 targets serine palmitoyltransferase (SPT), the enzyme catalyzing the first and rate-limiting step of sphingolipid biosynthesis. Inhibition was confirmed by sphingolipid profiling and U-$^{13}C_6$-glucose tracing, which showed a metabolic block at 3-ketosphinganine.

Experimental evolution under compound pressure yielded resistant strains carrying a single-nucleotide polymorphism in *LCB2*, encoding an essential SPT subunit. This mutation, resulting in an alanine-to-proline substitution, conferred resistance to Z247611722. Structural docking suggests that the compound interferes with the pyridoxal 5'-phosphate (PLP) binding site of SPT, acting via a distinct mechanism from the known inhibitor myriocin.

Z247611722 significantly reduced fungal burden in a *Galleria mellonella* infection model without showing any signs of toxicity, supporting its *in vivo* efficacy. As exogenous sphingolipids reduce its activity, topical formulations for oral, vaginal, or cutaneous candidiasis may be particularly promising.

These findings validate SPT as a druggable antifungal target and establish Z247611722 as a structurally distinct, novel inhibitor with therapeutic potential.

BLOCK A

Unravelling the impact of interferon-immunotherapy on epithelial resistance to *Candida albicans* translocation

Beatriz Cristóvão, Özlem Kirav, Marina Pekmezović, Saloni Saloni, Raquel Alonso-Román, Sophie Austermeier, Sascha Schäuble, Zoltán Cseresnyés, Marc Thilo Figge, Mark S. Gresnigt

HKI-Jena, Germany

Abstract

Invasive candidiasis is challenging to treat due to compromised immune system and the limited success of antifungal treatment alone. Immunotherapy has been posited as an approach to augment host defence of immunocompromised patients. Despite the beneficial role of the cytokine interferon gamma (IFN-γ), on augmenting myeloid antifungal activity, it remains unclear how an acute increase of IFN-γ levels influences intestinal epithelial cells that represent the first barrier against *Candida albicans*. Using an in vitro intestinal epithelial model, the association between fungal translocation, breakdown of epithelial barrier integrity and tight junction disassembly, upon acute treatment with IFN-γ was evaluated. We found that *C. albicans* translocation is increased upon IFN-γ treatment. Moreover, expression of junction proteins, are downregulated in the presence of IFN-γ. Accordingly, microscopy revealed disorganization of the tight-junction belt. We next exposed intestinal epithelial cells to the acute IFN-γ treatment during infection with *C. albicans* mutants lacking crucial virulence genes of. This included mutants deficient in the adhesin and invasin Als3 (*als3*D/D), the toxin candidalysin (*ece1*D/D), filamentation (*efg1*D/D/*cph1*D/D) as well as mutants with reduced proteolytic activity (*sap1/2/3*D/D & *sap4/5/6*D/D). Strikingly, fungal translocation of the *ece1Δ/Δ* and *als3*D/D mutants was also increased by acute exposure to IFN-γ. Though, *sap1/2/3*D/D and *sap4/5/6*D/D mutants showed no difference in translocation with IFN-γ exposure. Collectively, our data shows that reorganized barrier junctions following IFN-γ treatment make epithelial barrier more permissive to C. albicans translocation via damage-independent mechanisms, shedding a light on potential detrimental effects of interferon immunotherapy on barriers defence against *C. albicans* infection.

BLOCK A

Refining a Differentiated Gut Epithelium Model to Study Fungal-Bacterial Interactions

Megan T. Medrano, Kendall Esparrago, Paola Zucchi, Jesús A. Romo

University of Texas at San Antonio, USA

Abstract

Background: *Nakaseomyces glabratus* is an opportunistic fungal colonizer of the mammalian gut. Our group recently demonstrated that its presence exacerbates *Clostridioides difficile* infection (CDI) in a murine model, increasing both bacterial and toxin burdens. However, mechanisms underlying this exacerbation—particularly the role of fungal–bacterial interactions and host epithelial responses—remain unclear. A major limitation in dissecting these interactions is the lack of a standardized, human-relevant *in vitro* model.

Goal/Hypothesis: We aim to validate and characterize a differentiated Caco-2 epithelial model to study *N. glabratus–C. difficile* co-infection. We hypothesize that this system will recapitulate the disease exacerbation observed *in vivo* and provide mechanistic insight into how fungal colonization modulates CDI pathogenesis.

Methods: Caco-2 cells were seeded into transwells and differentiated over 21 days. Monolayer integrity and differentiation were assessed by LDH release, permeability assays (Lucifer Yellow, Dextran-FITC/TXRed), and qRT-PCR of key markers (*ZO1*, *EAAT1/3*, *PEPT1*, *SGLT1*, *GLUT1/2*). Differentiated monolayers were exposed to *N. glabratus* or media (control) and analyzed for damage and fungal burden at 6 and 24 hours post-infection.

Results: *N. glabratus* did not induce LDH release or increased permeability, indicating minimal epithelial damage. Fungal CFUs were recovered from apical, membrane, and basolateral compartments over time, consistent with asymptomatic colonization.

Future Directions: We are currently optimizing *C. difficile* infection parameters in this model, with planned co-infection studies to define how *N. glabratus* impacts CDI outcomes.

Conclusions: This model will enable mechanistic studies of cross-kingdom interactions driving gut disease severity.

BLOCK A

Delineating Genetic Determinants of Echinocandin Resistance in *Candidozyma* (*Candida*) *auris*

Garrett M. Weeks[1], Nicholas Cauldron[2], Katherine S. Barker[1], Jeffrey M. Rybak[1], Joachim Morschhäuser[3], Christina A. Cuomo[2], P. David Rogers[1]

[1]St. Jude Children's Research Hospital, USA. [2]Brown University, USA. [3]University of Wuerzburg, Germany

Abstract

Background. Echinocandin resistance has been reported to echinocandins in *C. auris*. In *C. albicans*, mutations in the gene encoding the echinocandin target confer resistance. Similar mutations have been observed in resistant *C. auris* clinical isolates. We aimed to determine the contribution of specific *FKS1* variants and to identify determinants of reduced susceptibility in isolates carrying *FKS1*^{WT}.

Methods. *FKS1* variants were identified from whole genome sequences of isolates with elevated micafungin (MCF) MICs among over 800 isolates. CRISPR-Cas 9 gene editing was used to introduce *FKS1* mutations in an echinocandin-susceptible isolate. Minimum inhibitory concentrations (MICs) for caspofungin (CPF), micafungin, and anidulafungin (ANF) were measured using modified CLSI methodologies. Genome-wide association studies (GWAS) were performed with *FKS1*^{WT} isolates with elevated MCF MIC.

Results. Among the *FKS1* variants observed were those leading to amino acid substitutions F635C, L636FL, F638F, S639F/P/Y, R641S, D642Y and M690V. Strains with these variants resulted in at least a two-dilution MIC increase for at least two echinocandins, except L638F and R641S with only elevated MCF MIC. Strains harboring F635C or the S639 substitutions reached or exceeded MCF and CPF tentative breakpoints set by CDC, while the L636FL strain only reached the CPF tentative breakpoint. Four genes were identified by GWAS as being associated with reduced echinocandin susceptibility in *FKS1*^{WT} isolates and are under further investigation.

Conclusions. Our findings demonstrate the effect of specific *FKS1* variants on echinocandin susceptibility, reveal the presence of non-*FKS1*-mediated resistance mechanisms, and point to potential non-*FKS1* echinocandin resistance determinants in *C. auris*.

BLOCK A

Candida albicans farnesol signaling mediated by Cwh8 as well as the farnesyl phosphate and pyrophosphate pools

Daniel Gutzmann[1], Cory Boone[2], Brigid Toomey[2], Shyanne Urbin[2], Wayne Riekhof[2], Deborah Hogan[1], Audrey Atkin[2], Kenneth Nickerson[2]

[1]Dartmouth College, USA. [2]University of Nebraska-Lincoln, USA

Abstract

In the opportunistic pathogen *Candida albicans*, hyphal growth and virulence factor expression are regulated by environmental and chemical cues. Farnesol is a secreted autoregulatory molecule that represses filamentation. It is derived from farnesyl pyrophosphate (FPP), an ergosterol biosynthesis pathway intermediate. Although Dpp1, Dpp2, and Dpp3 were proposed to synthesize farnesol, a mutant lacking all three had only a modest reduction in farnesol. To identify other farnesol biosynthesis genes, we employed mutants that underproduced or overproduced farnesol in a screen and analyzed their transcriptomes. *CWH8* was the only transcript correlated with farnesol production. Cwh8 is a lipid phosphatase known to recycle dolichyl pyrophosphate during glycosylation of cell wall proteins. The *cwh8ΔΔ* mutant had a >99% reduction in farnesol compared to the parent strain and was hyperfilamentous in embedded conditions. It had ~900-fold lower levels of farnesyl phosphate, the product of FPP dephosphorylation, suggesting Cwh8 acts directly on FPP. Complementation of the *C. albicans cwh8ΔΔ* mutant with *CWH8* from *C. albicans* restored cell wall defects and farnesol production. However, complementation with *CWH8* from *Clavispora lusitaniae*, a species lacking farnesol signaling, restored cell wall defects but failed to rescue farnesol production. This indicates that while Cwh8's role in maintaining cell wall integrity is conserved, differences in substrate specificity or interactions with species-specific cofactors underlie its involvement in farnesol biosynthesis in *C. albicans*. Finally, fluconazole was fungicidal for *cwh8ΔΔ* rather than fungistatic. These studies demonstrate how an extracellular signaling system can arise and be co-opted to promote fungal fitness in complex environments.

BLOCK A

Micafungin selects for multidrug resistance in *Clavispora lusitaniae* through *ERG3* mutations

Elizabeth Wash[1,2], Nancy Scott[2,3], Katura Metzner[2], Deveney Da Silva[4], Maurizio Del Poeta[4], and Anna Selmecki[1,2,3]

1. Genetics, Cellular and Developmental Biology, University of Minnesota
2. Microbiology and Immunology, University of Minnesota
3. Bioinformatics and Computational Biology, University of Minnesota
4. Microbiology and Immunology, Stony Brook University

Abstract

Non-*albicans Candida* species are increasingly responsible for candidiasis worldwide. A critical concern associated with this trend is their propensity for multidrug resistance which poses significant challenges for effective treatment options. We recently described the rapid evolution of a multidrug resistant *Clavispora lusitaniae* isolate from a patient undergoing micafungin monotherapy over an 11-day infection. The isolate acquired a single point mutation in *ERG3*, that corresponded to both micafungin and azole resistance, despite no patient history of azole therapy. We hypothesized that micafungin alone could select for multidrug resistance. Here, we determined the frequency and effect of mutations that arise during adaptation to micafungin treatment using parallel *in vitro* evolution experiments with the initial patient-derived, drug-sensitive *C. lusitaniae* isolate. Multiple independent mutations in genes of the ergosterol biosynthesis pathway (including *ERG3*), *FKS1*, and *OSH2* arose after exposure to micafungin *in vitro*. At least two independent mutations in each of these genes arose within different populations and reached high frequencies after ten passages, indicating a selective benefit. Relative to the progenitor, hotspot *FKS1* mutations conferred a growth advantage in micafungin; multiple distinct *OSH2* mutations conferred growth advantage in both fluconazole and micafungin; and distinct mutations in *ERG3* conferred growth advantages in micafungin, fluconazole, and amphotericin B. Subsequent deletion of *OSH2* and *ERG3* recapitulated drug resistance phenotypes and indicated the mutations acquired *in vitro* were loss of function. The *ERG3* mutants have unique cell wall and sterol composition, supporting that remodeling of the cell surface is sufficient to promote multidrug resistance. This study provides striking evidence that multidrug resistance can evolve in the presence of only a single echinocandin drug, both *in vitro* and during patient infection.

BLOCK A

Reduced Fluconazole Accumulation in *C. auris* Isolates with *ERG11* Mutations

Brooke Esquivel[1], DAVE ROGERS[2], THEODORE C WHITE[1]

[1]UMKC, USA. [2]ST. JUDE, USA

Abstract

We analyzed over one hundred *Candida auris* isolates with a variety of drug resistance profiles and representing each clade to gain a greater understanding of *C. auris* biology, antifungal resistance, and adaptations to stressful conditions.

We measured fluconazole import (drug accumulation in the absence of energy-dependent efflux), which revealed a very strong correlation between fluconazole susceptibility and drug accumulation. Fluconazole-resistant isolates have reduced fluconazole import compared to fluconazole-susceptible isolates.

Nearly all fluconazole-resistant *C. auris* isolates that have reduced fluconazole import have 1 of 3 specific *ERG11* variations compared to the *ERG11* sequence of susceptible isolates. In the rare cases of fluconazole-resistant isolates that have high import, the *ERG11* gene did not have the variant mutations.

Preliminary sterol analysis has shown ergosterol pathway aberrations in *C. auris* strains expressing variant *ERG11*. *ERG11* variant allele swaps in both resistant and susceptible parent isolates reversed the fluconazole MIC AND reversed the fluconazole **import** phenotype.

This suggests that variations in *ERG11* gene sequence not only results in fluconazole resistance directly by changing the azole drug target, but may also contribute to more complex, global cellular alterations such as increased/reduced levels of ergosterol, changes to membrane composition, fluidity, and local lipid and protein environments.

BLOCK A

Characterization of *CSA6*: A Novel Cell Cycle Regulator and Potential Antifungal Target

Mayssa Gnaien[1], Tyloran Benoit-Levy[1], Kaustuv Sanyal[2,3], Christophe d'Enfert[1], Matthijn Vos[1], Melanie Legrand[1]

[1]Institut Pasteur, France. [2]Jawaharlal Nehru Centre for Advanced Scientific Research, India. [3]Bose Institute, India

Abstract

Currently, clinicians have limited options to treat Candida infections, and the mortality rate from candidemia remains high with rising cases caused by drug-resistant fungal pathogens, reinforcing the urgent need for new antifungal molecules.

CSA6 was identified in a screen of ~1,000 overexpression mutants as an uncharacterized gene whose upregulation alters genome stability. It encodes an essential cell cycle factor localized to the spindle pole body (SPB). Sequence analysis shows *CSA6* is restricted to the CUG-Ser clade, which includes several major opportunistic fungal pathogens, but was initially believed absent from *Candida auris* and *Clavispora lusitaniae*.

Prompted by its apparent absence in these species, we conducted sequence analysis and identified candidate orthologs. Conditional depletion in *C. auris* confirmed its essentiality, and mutants are being analyzed for cell cycle defects. These results suggest Csa6 is a fast-evolving protein with conserved function and a promising clade-specific antifungal target.

The AlphaFold-predicted 3D model of Csa6 shows a long, high-confidence α-helix flanked by low-confidence regions. To assess its drug-target potential, structural and functional characterization is essential. To this end, we expressed and purified *C. albicans* and *C. auris* Csa6 in *Escherichia coli* and are optimizing a cryo-electron microscopy (cryo-EM) single-particle analysis (SPA) pipeline to resolve its structure.

Also, to investigate functionally relevant domains and identify potential interactors, we developed a screen for intra- and extragenic suppressor mutations alleviating toxic effects of *CSA6* overexpression/depletion.

Overall, our project aims to deepen our understanding of *C. albicans* biology - particularly SPB properties - and contribute to novel antifungal strategy development.

BLOCK A

Development of temporally and spatially resolved multidimensional reporter cell lines for host-fungal interactions

Verena Trümper[1], Jakob Sprague[1], Tim B. Schille[2,3], Myrto Katsipoulaki[2], Bernhard Hube[2,3,4], Sascha Brunke[2]

[1]Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knoell-Institute, Jena, Germany, Germany. [2]Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knoell-Institute, Jena, Germany. [3]Cluster of Excellence Balance of the Microverse, Friedrich Schiller University Jena, Germany. [4]Institute of Microbiology, Faculty of Biological Sciences, Friedrich Schiller University Jena, Germany

Abstract

Fungal infections have a tremendous impact on human health world-wide. Therefore, investigating fungal pathogenicity and fungal-host interactions at its basis are increasingly important. However, the chain of events during infections is complex, with a multitude of pathophysiological processes happening simultaneously on both sides. Understanding these requires many different and often time-consuming experimental procedures.

Thus, we are developing an easy-to-use, high-throughput method for temporally resolved screening of host cell responses. We have created multiple human cell lines originated from different body niches that can report, by activation of specific fluorophores, on different infection-associated intracellular events in parallel. These include, specifically, the activation of cell death pathways like apoptosis or changes in the redox environment of the cytosol or the mitochondria, which could be triggered by the pathogen *Candida albicans*. We use different fungal mutants to obtain additional information about cellular events during colonisation of the human body versus complex infection processes. As the reporter cell lines allow real-time measurements, they can give immediate insights into the kinetics of infections and host responses. The data generated will not only help to further decipher the network of underlying processes inside the host, but will also open new routes for further research into fungus-host interactions and pathogenicity.

In the future, this system can be applied not only to fungal infections, but also for dissecting bacterial and viral infections or drug-induced cellular responses. Collectively, this new assay will expand the current selection of methods available for fungal and infection biology studies.

BLOCK A

Regulation of Phosphatidylinositol-(4,5)-bisphosphate and Active-Rho1p Levels and Distribution is Crucial for Correct Spatio-temporal Cytokinesis and Echinocandin Responses in *Candida albicans*

Hassan BADRANE[1], M. Hong Nguyen[1], Cornelius J. Clancy[1,2]

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

Abstract

Candida species cause severe infections like invasive candidiasis, which annually affect 1.5 million people worldwide and cause close to 1 million deaths. *Candida albicans* is the predominant cause of candidiasis. We previously showed that EH domain-containing protein Irs4p binds 5-phosphatase enzyme Inp51p to regulate plasma membrane levels of phosphatidylinositol-(4,5)-bisphosphate ($PI(4,5)P_2$) in *C. albicans*. Indeed, deletion of *IRS4* or *INP51* led to elevated levels of $PI(4,5)P_2$ and presence of abnormal intracellular membranous $PI(4,5)P_2$ patches. To gain insights into the nature of these abnormal patches, we used fluorescent protein tagging and live cell imaging to follow their nascency. We show that these abnormal patches tightly correlate with cytokinesis, as they predominantly arise close to the site and time of cell division. We further demonstrate these patches colocalize $PI(4,5)P_2$ with actomyosin ring components Act1p and Myo1p, which form its core, and active Rho1p, a small GTPase that plays a regulatory role. Additionally, activation of Rho1p was altered in *irs4* and *inp51* mutants compared to wild-type strain, with over-activation or down-activation during early exponential or stationary phase, respectively. Wild-type cells exposed to 4xMIC of the echinocandin caspofungin show abnormal $PI(4,5)P_2$ patches colocalizing the same cytokinesis components as above, except that they were transient. Taken together, our results support a model in which $PI(4,5)P_2$ plays a pivotal role, along with Rho1p, in the correct execution of cytokinesis and response to caspofungin.

BLOCK A

Deepening the interaction of *Candida albicans* with phagocytes: Proteomic, phosphoproteomic and acetylomic studies on *C. albicans* recovery from oxidative stress.

Gloria Molero, Victor Arribas, Ana Borrajo, María Luisa Hernáez, Raquel Martínez, Lucía Monteoliva, Concha Gil

Complutense University of Madrid, Spain

Abstract

The limited antifungal arsenal available to treat invasive *Candida albicans* infections underscores the importance of discovering new antifungal targets. To identify novel proteins and processes critical for the survival of yeast within the host, our group investigated the recovery of *C. albicans* after 200 min of treatment with 10 mM H_2O_2 using proteomic, phosphoproteomic, and acetylomic approaches. Our study revealed the significant role of Prn1, a pirin-like protein with no homologs in *S. cerevisiae*, as well as Cub1, which was upregulated only when Prn1 was increased. Additionally, notable differences in the abundance of certain transcription factors, such as Mnl1 and Nrg1 (an antagonist of Mnl1), were observed between the *prn1Δ* and wild-type strains. We identified and quantified novel phosphosites in kinases and transcription factors. For example, reduced phosphorylation of Cdc5 was observed, which may mediate the transient G2 cell cycle arrest observed, whereas reduced Kis1 phosphorylation might be involved in reactive oxygen species (ROS) scavenging following oxidative stress. With regard to transcription factors, decreased phosphorylation of Gzf3 seems essential for cell survival and ROS detoxification after oxidative stress. Differences in protein acetylation involved proteins associated with ribosomes (translation and assembly), proton motor force-driven ATP synthesis, mitochondrial respiratory chain complexes, and the proteasome. Our work highlights the crucial role of proteomics in uncovering new proteins and post-translational modifications that are vital for the interaction of *C. albicans* with phagocytes, involving modifications other than MAPK phosphorylation or histone acetylation.

BLOCK A

Blood cultures (BCs) contain populations of genetically diverse Candida strains that may differ in antifungal resistance and tolerance

Fleres Giuseppe, Shaoji Cheng, Minh Hong Thi Nguyen, Cornelius Joseph Clancy

University of Pittsburgh, USA

Abstract

Background.

We showed that within-patient *Nakaseomyces glabrata* strains from BCs of 10 patients differed genetically, most strikingly by single nucleotide polymorphisms. In 2 patients, originally unrecognized fluconazole-resistant strains caused treatment failures. It is unknown whether within-patient BC diversity is common among other *Candida* spp.

Methods. We determined whole genome sequences of 10 *C. albicans* strains from BCs in each of 4 patients.

Results.

BCs in 3 patients contained *C. albicans* strains that differed by chromosome (Chr)5 or 7 aneuploidy (*n*=2) and Chr1 loss of heterozygosity (*n*=1). Tri7 *C. albicans* strains from patient M had impaired hyphal and biofilm formation in vitro compared to euploid strains, due at least partially to *NRG1* over-expression. Nevertheless, representative Tri7 strain M1 underwent filamentation during mouse disseminated candidiasis (DC). M1 was more fit than euploid strain M2 during DC and mouse gastrointestinal colonization, and in blood ex vivo. M1 and M2 exhibited identical echinocandin minimum inhibitory concentrations, but M2 was more tolerant to micafungin in vitro and more competitive with M1 in mouse kidneys following micafungin treatment than in absence of micafungin. Tri7 strains were 74% of the baseline BC population. After 3d of echinocandin treatment, euploid strains were 98% of the population. Findings suggest that echinocandin tolerant, euploid strains were a subpopulation to more virulent Tri7 strains at baseline and were selected upon echinocandin exposure.

Conclusions.

BCs in at least some patients are comprised of diverse *Candida* populations, including unrecognized antifungal-resistant or tolerant strains. Clinical relevance of *Candida* diversity merits further investigation.

BLOCK A

Circumventing drug resistance: from identifying new drug resistance markers to developing tools for drug target discovery

Miguel Teixeira[1], Diogo Couceiro[1,2], Pedro Monteiro[2,3], Romeu Viana[1], Inês Costa[1], Maria Zolotareva[1], Madalena Silva[1], Luís Coutinho[1]

[1]Department of Bioengineering and iBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Portugal. [2]INESC-ID, Portugal. [3]Department of Computer Science, Instituto Superior Técnico, Universidade de Lisboa, Portugal

Abstract

Identifying new drug targets and the development of new drugs is crucial to overcome current increase in therapeutic failure, especially for non-albicans Candida species.

Our efforts in the identification of new pathways underlying antifungal drug resistance have led us to uncover the role of several new drug transporters involved in the efflux, but also uptake of azole drugs. Particularly, in silico evolution, followed by genome sequencing, enabled the identification of a new role for hexose transporters in azole resistance, leading us to propose that at least *Candida glabrata*Hxt4/6/7 and Hxt6/7 may constitute a family of azole importers [Galocha et al, Commun Biol, 5(1):1118, 2022]. In silico analysis is being used to assess hexose transporters' integrity and loss of function as a potential key differentiator between azole susceptible and resistant clinical isolates.

In an effort to identify new drug targets, we are building and validating genome-scale metabolic models (GCMMs) for pathogenic *Candida* and *Cryptococcous* species [Viana et al, J Fungi. 2020, 6:171; Viana et al, Genes. 2022, 13:303; Viana et al, FEMS Yeast Res. 2023, 23:foad045], and combining them with regulatory information gathered in the YEASTRACT database [Teixeira et al, NAR. 2023 51:D785-D791]. The mixed regulatory-metabolic models under construction will be used as platforms to identify promising new drug targets by determining enzyme essentiality in conditions mimicking the human host.

Altogether, both approaches provide new ways to address drug resistance, while tackling human fungal infections.

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BLOCK A

Engineered Candidalysin-Binding Proteins: A Versatile Platform for Therapeutic and Diagnostic Applications in *Candida* Infections.

Maros Huliciak[1], Azam Khedri[1], Landon Burton[1], Shankar Thangamani[2], Eric Sundberg[1], Sohail Khoshnevis[1]

[1]Emory University School of Medicine, USA. [2]Purdue University, USA

Abstract

Background: Invasive *Candida* infections are a significant cause of morbidity and mortality, particularly in immunocompromised individuals. *Candida albicans*, the most common pathogenic species, secretes candidalysin, a peptide toxin essential for epithelial damage, immune activation, and fungal virulence. Neutralizing candidalysin offers a promising therapeutic strategy to mitigate tissue damage and excessive inflammatory responses during infection. However, the development of specific biologics agents targeting candidalysin remains unexplored. To address this critical gap and test the hypothesis that a neutralizing protein targeting candidalysin will inhibit its cytolytic activity and reduce epithelial damage and pro-inflammatory cytokine production, we developed and characterized candidalysin-binding proteins (CLBs) to detect and neutralize candidalysin *in vitro* and tested their efficacy and toxicity *in vivo*.

Results: We created combinatorial libraries of protein scaffolds and used ribosome display method to select for specific binding to candidalysin toxin. Several protein variants with highest affinity to candidalysin were selected. We showed that these proteins are very stable and bind to candidalysin in a concentration-dependent manner. These CLBs can neutralize candidalysin-mediated epithelial damage and signaling without adversely affecting the host. In addition to therapeutic application, we also established CLBs as a diagnostic tool to detect candidalysin. Using biophysical methods, we uncovered the mode of neutralization of candidalysin by CLBs.

Conclusions: Our strategy to neutralize *Candida* infection by targeting candidalysin with engineered small proteins represents a novel therapeutic avenue that mitigates tissue damage and inflammation. By disarming, rather than killing the pathogen, this approach preserves the healthy microbiota and minimizes the risk of antifungal resistance.

BLOCK A

Distribution of Candida Species and Antifungal Susceptibility Pattern among HIV Positive Individuals with Oropharyngeal Infection in Selected Mekelle Health Facilities, Tigray, Northern Ethiopia

MAHMUD Shukur

Haftay, Ethiopia

Abstract

In recent time there is a change in spectrum of *Candida* species as causation agent of oropharyngeal candidiasis and increased antifungal drug resistance among HIV infected. to determine distribution and antifungal susceptibility pattern *Candida* species among HIV positive oropharyngeal patients. A cross-sectional study conducted among 381 HIV positive oropharyngeal infection. swab samples cultured on Sabouraud, germ, HiCrome agar. Antifungal susceptibility patterns Candida species determined.The overall *Candida* species isolation rate 59.8% (228/381). *C. albican* most predominant species 151(62.9%). Followed *Candida g*, *Candida t*, *Candida k*. previous history of antifungal drug treatment, CD4 counts < 200 cells/mm3, HAART, body mass indices <15.9 kg/m2, WHO clinical stage III, TB co-infection significantly associated with *Candida* species isolates. Antifungal susceptibility testing was performed on all *Candida* species isolates and 12.9% and 9.2% of them showed resistance to fluconazole and ketoconazole respectively. Least rate of resistance was against nystatin 0.8%. **Conclusion:**Non-albican *Candida* species drug resistance to azole groups increasing. Nystatin less resisted antifungal agent, early identification etiologic agent and antifungal susceptibility testing important

Key words: *Oropharyngeal, Candidiasis, Candida species distribution, Antifungal susceptibility, HIV, Mekelle, Ethiopia*

BLOCK A

The presence of Candida on human epithelial tissues can influence the memory function of the innate immune system

Zsolt Tasi[1], Ádám Novák[1], Máté Lajos Csikós[1], Attila Gácser[1,2,3,4]

[1]Department of Biotechnology and Microbiology, University of Szeged, Hungary. [2]HCEMM-USZ Fungal Pathogens Research Group, Hungary. [3]HUN-REN-USZ Pathomechanisms of Fungal Infections Research Group, Hungary. [4]University of Szeged, IKIKK, Competence Centre for Molecular Biology, Bionics and Biotechnology, Hungary

Abstract

Keratinocytes, as part of the innate immune system, play a vital role in maintaining the skin's protective barrier. As the outermost layer of the body, keratinocytes frequently encounter various microorganisms and respond to the presence of pathogens, including bacteria, fungi, and viruses. During such innate immune responses, innate immune memory also often develops. This is established through specific epigenetic and metabolic changes that develop during the repeated interactions with microbes, enabling a more robust immune response.

Here we aimed to investigate the effect of the various fungal components of the integument(Candida albicans and Candida parapsilosis) on the development of innate immune memory. For the experiments two clinical isolates of two different Candida species were used as pathogens (C. parapsilosis GA1 and CLIB214; C. albicans WO1 and SC5314), and two different keratinocyte cell lines (HaCaT and HPV-KER) were applied representing the skin outer layers, as the host. After the pre-treatment of HaCaT/HPV-KER cells with C. parapsilosis cells for 24hours, host cells were immediately challenged with C. albicans cells for 24hours. Then, ELISA and LDH assays were used to determine keratinocyte cell responses. Our results indicate that pre-treatment with both C. parapsilosis strains significantly decreases the extent of host cell damage in both HaCaT and HPV-KER cells (from approx. 20% to >10%, based on LDH release) and elevate inflammation via increasing IL-6 and IL-8 levels. Besides priming, immune-training is another mode of innate immune memory development. Next, we aim to examine memory formation via immune memory using the above-mentioned species.

BLOCK A

Candida parapsilosis CPAR2_702260: A linkage between sulfur metabolism and temperature tolerance

Dominika Flaisz[1], Tibor Németh[1], Mátyás Selmeci[1], Attila Gácser[2,3,4]

[1]Department of Biotechnology and Microbiology, University of Szeged, Hungary. [2]HCEMM-USZ Fungal Pathogens Research Group, Department of Microbiology, Szeged, Hungary. [3]HUN-REN-SZTE Pathomechanisms of Fungal Infections Research Group, University of Szeged, Hungary. [4]University of Szeged, IKIKK, Competence Centre for Molecular Biology, Bionics and Biotechnology, Hungary

Abstract

Candidiasis represents a severe medical challenge even in developed countries. In recent decades, in addition to *C. albicans*, *C. parapsilosis* has received an increasing attention, due to its rising incidence particularly in clinical settings. The adaptation of the pathogen to the ever-changing environmental conditions is essential for its survival, making stress tolerance an important aspect in its virulence. *Candida* cells can be exposed to various types of stress in the host out of which the most important one is temperature. Based on the previous studies in our laboratory, the deletion of the *CPAR2_702260* ORF of *C. parapsilosis* resulted in a strong growth defect when the cells were cultivated at 37 °C. The ortholog of this gene in *Saccharomyces cerevisiae* is *MET28* that encodes a transcriptional regulator of the sulfur metabolism. In *S. cerevisiae*, the absence of *MET28* disrupts the ability of the cells to utilize inorganic sulfur. Further characterization of the *CPAR2_702260* KO mutant revealed a similar phenotype at 30°C which could have been complemented with methionine but not with cysteine. Interestingly, the growth defect observed at 37 °C could have been complemented with sorbitol, NaCl, EDTA, and methionine as well. Based on our results, we concluded that *CPAR2_702260* plays an important role in the temperature tolerance and the sulfur metabolism. This project was funded by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the FK 22 funding scheme, project no. 143373.

BLOCK A

The Genetic Background modulates the proteome response to RPD3L complex genetic perturbations

Alexis García[1], Julia Muenzner[1], Jing Hou[2], Pauline Trebulle[1], Markus Ralser[1]

[1]Charité Universitätsmedizin, Germany. [2]University of Strasburg, France

Abstract

The genetic background effect, interactions between genes that influence another gene's function, complicates efforts in personalized medicine, genetic counseling, predicting antimicrobial resistance. This study examines how genetic background alters proteomic responses in Saccharomyces cerevisiae.

Ten genetically diverse strains from the 1011 Yeast Isolates Collection (Peter et al., 2018) were subjected to eight gene knockouts targeting subunits of the RPD3L histone deacetylase complex, which represses early meiosis and metabolic genes under favorable conditions (Patel et al., 2023). Genes were selected for strong proteomic response and knockout fitness (Messner et al., 2023). CRISPR/Cas9 and Synthetic Genetic Arrays (SGA) were used for perturbations. Proteomes were profiled using DIA mass spectrometry, detecting aproximately 3300 proteins. Strain-specific variability was observed, potentially due to differences in RPD3L degradation patterns and variation in promoter binding motifs, highlighting how genetic background shapes gene function.

Messner, C. B., Demichev, V., Muenzner, J., Aulakh, S. K., Barthel, N., Röhl, A., Herrera-Domínguez, L., Egger, A.-S., Kamrad, S., Hou, J., Tan, G., Lemke, O., Calvani, E., Szyrwiel, L., Mülleder, M., Lilley, K. S., Boone, C., Kustatscher, G., & Ralser, M. (2023). The proteomic landscape of genome-wide genetic perturbations. *Cell*, 2018–2034. https://doi.org/10.1016/j.cell.2023.03.026

Peter, J., De Chiara, M., Friedrich, A., Yue, J.-X., Pflieger, D., Bergström, A., Sigwalt, A., Barre, B., Freel, K., Llored, A., Cruaud, C., Labadie, K., Aury, J.-M., Istace, B., Lebrigand, K., Barbry, P., Engelen, S., Lemainque, A., Wincker, P., … Schacherer, J. (2018). Genome evolution across 1,011 Saccharomyces cerevisiae isolates Species-wide genetic and phenotypic diversity. *Nature*.

BLOCK A

Exploring the role of the tRNA epitranscriptome in *Candida albicans* pathogenicity

Louise Abreu[1,2], Ana Poim[1], Beatriz Rebelo[1,2], Rafaela Beleza[2], Maria João Carvalho[1], Ana Raquel Soares[1], Ana Rita Bezerra[1]

[1]Institute of Biomedicine, Department of Medical Sciences, University of Aveiro, Portugal. [2]Department of Biology, University of Aveiro, Portugal

Abstract

In recent years, the tRNA epitranscriptome has emerged as a promising therapeutic target to tackle infections. As key translation effectors, tRNAs ensure efficient and accurate mRNA codon recognition for proper protein synthesis. tRNA modifications, that are catalyzed by different classes of tRNA modifying enzymes, are also essential for aminoacyl tRNA synthetase binding and amino acid charging, while maintaining tRNA function and structure. A single molecule of tRNA can have an average of 13 different modifications that are highly dynamic in response to environmental cues. The rapid reprogramming of tRNA modifications in response to stress is essential to fine-tune translation and promote the preferential translation of stress response genes, which is an essential trait for adaptation in various pathogens. tRNA modifications have also been identified as virulence regulators in different pathogens, including bacteria, plasmodium, and fungi. However, the contribution of tRNA epitranscriptomic regulation to *Candida albicans* pathogenicity remains largely unexplored. In this study, we analyzed 241 clinical *C. albicans* isolates (iBiMED-UA biobank), assessing virulence-associated phenotypes including antifungal susceptibility, morphogenesis, biofilm formation, and stress resistance. Whole-genome analysis identified point mutations in 36 genes predicted to encode tRNA-modifying enzymes. Furthermore, preliminary data indicate inter-strain variability in both tRNA modification levels and expression of modifying enzymes, which correlates with phenotypic virulence markers. These findings suggest that the *C. albicans* tRNA epitranscriptome is a key determinant of virulence and pathogenicity and that targeting the tRNA epitranscriptome can constitute a novel antifungal strategy.

BLOCK A

Antifungal Activity of Moringa oleifera and Zanthoxylum zanthoxyloides Against Clinical Candida Species: Phenotypic and Molecular Characterization, Resistance Profiling, and Synergy Analysis

Olorunjuwon Bello[1], Temitope Bello[2], Bridget Akinpeloye[1]

[1]University of Medical Sciences, Ondo City, Ondo State, Nigeria. [2]Eliizade University, Ilara-Mokin, Ondo State, Nigeria

Abstract

The increasing resistance of *Candida* species to conventional antifungal drugs poses a significant clinical challenge. With the limitations of existing therapies, there is a growing interest in identifying plant-derived compounds with antifungal properties. This study investigates the antifungal activity of *Moringa oleifera* and *Zanthoxylum zanthoxyloides* against clinical *Candida* isolates. A total of 120 clinical *Candida* were obtained from two teaching hospitals. Re-identification of the isolates was performed using germ tube tests, CHROMagar, and PCR technique. Antifungal susceptibility was assessed using the broth microdilution method with fluconazole, ketoconazole, and amphotericin B. Leaves of *M. oleifera* and roots of *Z. zanthoxyloides* were extracted using methanol, and water. Standard phytochemical tests screened for alkaloids, flavonoids, tannins, phenolics, and saponins. Antifungal activity was evaluated using agar well diffusion and broth microdilution assays, while synergy with antifungal agents was assessed using the checkerboard method and interpreted using the fractional inhibitory concentration index (FICI). *C. albicans* (40%) was most prevalent, followed by *C. glabrata* (23.3%), *C. tropicalis* (20.8%), and *C. krusei* (15.9%). Resistance to fluconazole was highest in *C. krusei* (82%) and *C. glabrata* (58%). Bioactive compounds were revealed in both plants, with saponins detected only in *M. oleifera*. *Z. zanthoxyloides* exhibited higher zones of inhibition than *M. oleifera*. Checkerboard assays indicated synergistic interactions (FICI ≤ 0.5) between both plant extracts and fluconazole, with additive effects observed with ketoconazole. *M. oleifera* and *Z. zanthoxyloides* demonstrated promising antifungal activity against resistant *Candida* species. Their synergistic effects with standard antifungals support their potential as complementary therapeutic agents.

BLOCK A

The evolution of antifungal drug tolerance in clinical isolates of *Candida albicans*

Evangelina Krasteva

Charite - Berlin University of Medicine, Germany

Abstract

Opportunistic pathogens such as *Candida albicans* typically cause little harm to healthy individuals but can lead to severe infections in immunocompromised hosts. Among antifungals, fluconazole is widely used due to its pharmacological profile and broad activity. However, prolonged exposure selects for both resistance and tolerance. Resistance involves genetic adaptations that directly affect the drug target and/or intracellular drug concentrations that permit growth at elevated drug concentrations. Nevertheless, tolerance enables survival at supra-MIC levels without altering the MIC itself, and thus may evade detection in routine assays, contributing to treatment failure and resistance development. However, the development of tolerance and its role in acquiring resistance is still unclear. To investigate that, clinical *Candida* isolates with varying tolerance levels were serially passaged under fluconazole pressure using disc diffusion assay. Results showed that tolerance levels increased gradually, while changes in resistance were less pronounced and strain-specific. In some isolates, a distinct colony phenotype associated with heteroresistance emerged, allowing for further insight into the evolutionary pathway. The stability of these traits was demonstrated by removing antifungal pressure, followed by re-exposure to fluconazole. Future work includes infecting *Galleria mellonella* with evolved and parental strains to assess pathogenicity and fungal burden in vivo. Proteomes and whole genomes of evolved strains will be analyzed to gain insights into the causes and dynamics of tolerance emergence. This study investigates the evolution of tolerance and combines multi-omics approaches to uncover the mechanisms underlying its emergence and progression, ultimately providing a foundation for strategies to mitigate its clinical impact.s

BLOCK A

Characterisation of *Candida albicans* biofilm extracellular vesicles to inform antifungal strategies

Precious Kelebogile Letebele, Carolina H Pohl, Jacobus Albertyn

University of the Free State, South Africa

Abstract

The publishing of the World Health Organisation's first Fungal Priority Pathogen List (WHO FPPL) critical group emphasises the significant level of public health impact of fungal pathogens and the need to develop new and improved diagnostic tools, treatments, and preventive measures for these pathogens. Extracellular vesicles (EVs) are nanoparticles composed of lipid bilayered membrane structures, considered "virulence bags," which carry several biomolecules, including proteins and lipids. Depending on the fungal pathogen, EVs can interact with the host immune system to elicit multiple outcomes and exacerbate or attenuate fungal infections. Here, focus on *Candida albicans* and its lipid metabolism-related gene, *EHT1*. We employed a CRISPR-Cas9 system to construct a homozygous mutant of *EHT1* and performed RNA sequence analysis to identify the differentially expressed genes in mutant vs. wild-type biofilms. Furthermore, we evaluated the effect of the deletion on *C. albicans* growth, response to stress, hyphal formation, and, importantly, EV production and cargo. Our results show an overlap of the whole cell transcriptomics and EV protein cargo, emphasising that the content of EVs represents the metabolic state of the cells from which they were derived. In addition, our multi-omics results indicate that genes involved in lipid metabolism play a role in key virulence factors such as biofilm hyphal formation, cell wall organisation and EV production in *C. albicans*. These findings provide insight into biofilm pathogenic mechanisms and opportunities for the development of antifungal therapies.

BLOCK A

Cbk1 Integrates Polarity and Cell Cycle Control to Maintain Hyphal Growth

Encarnación Dueñas[1], Patricia Rojo[2], Soledad Píriz-Antúnez[2], Antonio Esperilla-Muñoz[2], Nuria Trevijano-Contador[2], Toni Ciudad[2], Jaime Correa-Bordes[2], Carlos R Vázquez de Aldana[1]

[1]Instituto de Biología Funcional y Genómica. USAL-CSIC, Spain. [2]Dpto. Ciencias Biomédicas. Universidad de Extremadura, Spain

Abstract

Hyphal growth in *Candida albicans* is critical for pathogenicity. The NDR kinase Cbk1 promotes hyphal initiation, but its role in maintenance was unclear. Using a *cbk1-as* mutant, we show that Cbk1 activity is essential for sustained apical elongation after septin ring assembly. Time-lapse fluorescence microscopy of secretory pathway and polarisome markers revealed that Cbk1 maintains secretion at the hyphal tip independently of the cell cycle. Cbk1 also regulates activation and tip localization of the polarity regulator Cdc42, with a putative Cbk1 phosphorylation site in Cdc42 being crucial for robust hyphal growth. Additionally, Cbk1 enforces cell cycle arrest in subapical compartments, preventing lateral budding. Inhibition of Cbk1 during maintenance led to reactivation of the cell cycle in subapical cells, which correlated with an illicit accumulation of Ace2 in these cells and an increase in the expression of the G1 cyclins *CCN1*, *PCL1*, and *PCL2*. Deletion of *ACE2* significantly suppressed lateral budding in the *cbk1-as* background. These results position Cbk1 as a central regulator of *C. albicans* filamentation, integrating polarity maintenance, Cdc42 activation, and cell cycle control to sustain polarized growth and suppress lateral branching.

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BLOCK A

Azole-resistant cytoplasmic translation mutants in *Candida glabrata* have similar fitness and resistance profiles as *PDR1* gain-of-function mutants

Vladimir Gritsenko, Anastasia Varnavski, Shada Abu Tayeh, Alona De Russo, Farhan Feroze, Judith Berman

Shmunis School of Biomedical and Cancer Research, Tel Aviv University, Israel

Abstract

Fluconazole resistance emerges rapidly in *Candida glabrata,* the second most common cause of invasive fungal infections in Western countries, and is primarily mediated by the master transcriptional regulator Pdr1. Gain-of-function mutations in *PDR1*, which upregulates the Cdr1 efflux pump, are a main focus of current literature. Here, we evolved a large collection of fluconazole-resistant mutants in five different clinical backgrounds, and systematically characterized the non-petite adaptors. In addition to *PDR1* mutants, we found a large proportion of mutations in proteins involved in cytoplasmic translation, including structural ribosomal components, ribosome biogenesis factors and assembly factors; missense and nonsense mutations predominated, suggesting that loss-of-function alleles increased fluconazole resistance directly or indirectly. Consistent with this, deletion of two different ribosome biogenesis factors increased resistance to fluconazole in several different strain backgrounds. Resistance and fitness profiles of the translation mutants had similar growth rates and maximum O.D, but took longer to exit lag phase relative to *PDR1* mutants based on fluconazole dose-response curves. Importantly, in the translation mutants, as in *PDR1* mutants, most resistance was lost when *CDR1* was deleted, implying that cytoplasmic translation mutants rely upon Cdr1 activity to confer fluconazole resistance. This insight aligns with a recent transposon mutagenesis report (Gale *et al*., 2023) that cytoplasmic translation is a major mechanism of azole resistance which acts via Cdr1 efflux activity. We posit that reduced cytoplasmic translation, like loss of mitochondrial function, indirectly triggers stress responses that activate drug efflux pumps.

BLOCK A

SF1: a new marine natural compound with potent antifungal activity

Susana Ribeiro, Nuno M. Saraiva, Cláudia Malta-Luís, Teresa Pissarro, Américo G. Duarte, Oscar L. Rojas, M. Rita Ventura, Catarina Pimentel

ITQB NOVA, Portugal

Abstract

Invasive fungal infections (IFIs) have been increasing in recent years, causing substantial morbidity and mortality. Current therapeutic strategies rely predominantly on three systemic antifungal classes: azoles, polyenes, and echinocandins. However, rising resistance to these agents has significantly reduced their clinical efficacy, underscoring the urgent need for novel antifungal therapeutics with distinct mechanisms of action.

We have recently identified a potent antifungal compound derived from a marine sponge extract, designated SF1. SF1 exhibits strong efficacy against several *Candida* species responsible for IFIs, including those inherently tolerant to azoles such as *C. glabrata* and *C. krusei*. It is fungicidal, capable of overcoming resistance to existing antifungals, and acts through a novel mechanism of action. Furthermore, SF1 addresses gaps in the current antifungal pipeline, showing activity against species such as *C. krusei* and *C. guilliermondii*. To ensure a scalable and sustainable supply, efforts are currently underway to establish a synthetic route for SF1.

BLOCK B

Blocking the shikimate pathway amplifies the impact of carvacrol on biofilm formation in *Candida albicans*

Ali Molaeitabari, Tanya E. S. Dahms

Department of Chemistry and Biochemistry, University of Regina, Regina, Saskatchewan, Canada

Abstract

Candida albicans typically thrives in a commensal relationship with humans but is also an opportunistic fungal pathogen. As an opportunistic pathogen, *C. albicans* relies heavily on its ability to assimilate nutrients, for which it must compete with the host and other microorganisms. Amino acid biosynthesis, sensing, and uptake play pivotal roles in *C. albicans* growth and pathogenicity. *C. albicans* biosynthesizes aromatic amino acids and co-enzyme Q de novo through the shikimate pathway, including the Aro1, Aro2, and Aro7 enzymes, but also has amino acid transporters for uptake from the environment. Thus, antifungal approaches targeting aromatic amino acid biosynthesis must simultaneously inhibit amino acid biosynthesis and uptake. Herein, we investigate the plant-based antifungal, carvacrol, in conjunction with aromatic amino acid biosynthetic mutants, as a potential anti-candidal strategy. Growth of the WT, *ARO2*, and *ARO7* strains were inhibited by 150 µg/mL carvacrol, whereas the *ARO1* mutant was slightly more sensitive (with MIC 125 µg/mL). All repressed mutants exposed to carvacrol are partially rescued in the presence of para-aminobenzoic acid (PABA) (CoQ precursor), indicating that blocking the shikimate pathway impacts both aromatic amino acid and CoQ biosynthesis. Moreover, carvacrol at sublethal concentrations significantly inhibits *ARO1* adhesion and hyphal formation, along with pre-attached and pre-formed hyphae, ultimately impacting biofilm metabolic activity and biomass accumulation and significantly reducing biofilm growth. In summary, carvacrol increases the sensitivity of *C. albicans* to *ARO1* repression, with attenuated adhesion, hyphal formation, mycelial growth and biofilm formation, likely by blocking aromatic amino acid uptake.

BLOCK B

Wall incorporation of the β-1,3-glucan crosslinking protein Pir1 in *Candida albicans* is facilitated by two or more pir repeat units

María Alvarado[1], Ana Esther Moreno-Martínez[1], Jesus Alberto Gomez-Navajas[1], Sebastian Fernandez-Sanchez[1], Maria Teresa Blázquez-Muñoz[1], Toni Gabaldon[2], Estibaliz Mateo[3], Eulogio Valentín[4], Piet W. J. de Groot[1]

[1]ETSIAMB, University of Castilla-La Mancha, Spain. [2]Barcelona Supercomputing Centre (BSC-CNS), Spain. [3]Department of Immunology, Microbiology and Parasitology, University of the Basque Country, Spain. [4]Severe Infection Research Group, Medical Research Institute La Fe, Valencia, Spain, Spain

Abstract

The cell wall of *Candida albicans* plays a crucial role in maintaining structural integrity and protecting the cell from environmental stress, while also acting as the primary interface in host-pathogen interactions. Among its components, Pir (proteins with internal repeats) protein family members are believed to stabilize the wall by forming multiple covalent bonds with β-1,3-glucans via their repeat units. *C. albicans* encodes two *PIR* genes: ***PIR1***, which encodes for an alkali-sensitive wall protein with **multiple internal repeats**, and ***PIR32***, which has only a **single repeat** and has never been detected in the cell wall. Previous studies using *PIR* deletion mutants have yielded conflicting results regarding their roles in cell wall integrity, prompting us to explore their functions in more depth. We found that only ***pir1Δ/Δ*** mutants displayed **mild cell wall-related phenotypes**, notably a **reduced** ability to **bind laminarin**, consistent with its proposed function in β-1,3-glucan interaction. Mutagenesis experiments revealed that at least **two repeat units are required** for covalent **incorporation** of Pir1 into the cell wall and for its proper **functionality**. Structural modeling supports this, showing Pir1 as having a core of anti-parallel β-sheets with an external loop containing all but one of the repeat units. Site-directed mutagenesis of specific amino acids within these repeats confirmed their essential role in cell wall integration. In summary, our work provides new mechanistic insights into how Pir1 is anchored to the cell wall, supporting its function in reinforcing the glucan network of the critical pathogen *C. albicans*.

BLOCK B

Does fungal ageing impact on *Candida albicans* ability to cause disease and development of antifungal resistance?

Johanna Gosciniak[1], Eloise Mitchell[2], Neil Gow[1], Alessandra da Silva Dantas[2]

[1]MRC CMM - University of Exeter, United Kingdom. [2]Newcastle University, United Kingdom

Abstract

Opportunistic systemic fungal infections, such as those caused by *Candida spp.*, can arise in immunocompromised patients, and are associated with high mortality rates that is potentiated by antifungal drug resistant infections. Several pathways have been identified as key regulators of antifungal resistance in *Candida albicans* in cultures composed predominantly of young cells. However, replicatively aged fungal cells have been reported to be enriched during *Candida* infections, suggesting that 'older' cells are better equipped to survive in the host environment. Hence, the aim of this work is to characterise how replicative ageing changes *C. albicans* physiology and to evaluate the contribution of **R**eplicatively **AGE**d (**RAGE**) cells to pathogenesis and antifungal resistance. To study replicative ageing in *C. albicans* a novel "track and trace" methodology has been developed, where we can **track** *C. albicans*RAGE cells and **trace** offspring. RAGE cells display some of the characteristic ageing hallmarks seen in other organisms, such as increased levels of mitochondrial reactive oxygen species and changes in chitin, glucan and mannan levels. Importantly, we have found that RAGE cells demonstrate tolerance to antifungal drugs and phagocytic mediated killing when compared with young cells. This data supports a model where RAGE cells contribute to survival of *C. albicans* in the host and in the emergence of antifungal drug resistance. Current work is directed at understanding the physiological basis of stress tolerance in RAGE cells, and to explore mechanisms to subvert replicative ageing as an antifungal strategy against *Candida* infections.

BLOCK B

Profiling of compound-induced cellular effects via high-throughput imaging and machine learning to gain early MoA insights

Jonathan Ruwe[1], Oliver Aehlig[1], Matthias Fischer[1], Timo Leistner[1], Pierre Stallforth[2,3], Luzia Gyr[1]

[1]Junior Research Group Robotics-assisted Discovery of Antiinfectives, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany. [2]Department of Paleobiotechnology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany. [3]Institute for Organic and Macromolecular Chemistry, Faculty of Chemistry and Earth Sciences, Friedrich Schiller University Jena, Germany

Abstract

The increased occurrence of resistant fungal pathogens and the lack of new antifungal agents reaching the market pose a significant threat to human health.

Basic phenotypic screens can rapidly identify promising compounds based on growth inhibition, but typically require extensive follow-up experiments to determine whether a compound acts through a novel mode of action (MoA). To address this limitation, we are establishing a high-throughput image-based profiling assay that can provide first insights into compound MoAs. This approach leverages our modularized robotics platform, JenXplor, enabling parallel testing of large compound libraries and prioritization of compounds with potentially new MoAs.

The assay utilizes fluorescent dyes to label key cellular substructures, enabling the observation of compound-induced morphological changes using high-throughput microscopy. These images are analyzed using a machine learning model extracting image features to cluster distinct phenotypes that can be attributed to specific MoAs, thus identifying compounds with potentially novel ones.

Historically, natural products have been a rich source of bioactive compounds in drug discovery, in part due to evolutionary selection for bioactivity, making them well-suited as test objects for our approach. We screened an in-house natural product library of over 9000 compounds against *Candida albicans,* yielding more than 70 potential hits. Their activities were determined via minimum inhibitory concentration assay, identifying several compounds with sub-micromolar activity. These promising compounds are now undergoing profiling to gain insights into their MoA.

This approach facilitates the rapid discovery and prioritization of antifungal compounds with potentially novel MoAs, accelerating their progression into the drug development pipeline.

BLOCK B

Lifespan Diversity in *Candida*: Insights Between and Within Species

Alberto Luna-Carrera[1,2], Alexander DeLuna[1], Eugenio Mancera[2]

[1]Centro de Investigación sobre el Envejecimiento, CINVESTAV, Mexico.
[2]Departamento de Ingeniería Genética, CINVESTAV, Unidad Irapuato, Mexico

Abstract

In microorganisms, including those that inhabit the human body and cause infections, adverse environmental conditions often trigger entry into a non-dividing, stationary phase that enables prolonged survival and re-entry into proliferative state. In yeasts, the duration during which cells remain metabolically active and viable in this state is known as the chronological life span (CLS). Despite its clinical relevance, the CLS of *Candida* species remains poorly understood. In this study, we used standard and high-resolution experimental approaches to characterize the CLS across eight *Candida* species, including clinically prevalent pathogens such as *C. albicans* and *C. auris*, as well as species less commonly associated with infections like *C. sojae* and *C. maltosa*. Our analysis revealed substantial interspecific variation in life span and significant longevity shifts under caloric restriction. Notably, lifespan did not correlate with pathogenicity across species, as both short- and long-lived strains were found among human-associated *Candida*. However, we observed strong intraspecific association between longevity and virulence emerging in a panel of twenty *C. albicans* strains with previously reported virulence profiles. Overall, CLS showed no clear correspondence with phylogenetic relationships, suggesting that stationary-phase survival mechanisms evolved independently of virulence traits. By performing large-scale analyses of deletions of orthologous transcriptional regulators involved in CLS modulation, we are starting to shed light on mechanisms underlying high interspecific variation of CLS in *Candida*. Together, these findings provide new insight into the adaptive strategies and stress resistance of *Candida* species.

BLOCK B

Evolutionary trajectories of aneuploidy adaptation in natural isolates of Candida albicans

Julia Muenzner[1], Lisa Kimberly Henning[1], Judith Berman[2], Markus Ralser[1,3,4]

[1]Charité University Medicine Berlin, Germany. [2]Tel Aviv University, Israel. [3]The Wellcome Center for Human Genetics, Nuffield Department of Medicine University of Oxford, United Kingdom. [4]Max Planck Institute for Molecular Genetics, Germany

Abstract

Candida albicans exhibits high genome plasticity, with aneuploidy - an imbalance in chromosome copy numbers - being well tolerated. Acquired aneuploidy has been linked to the emergence of both drug resistance and tolerance in *Candida albicans*, but the dynamics and mechanisms of aneuploidy adaptation remain poorly understood. In *Saccharomyces cerevisiae*, we found that protein turnover is linked to the attenuation of gene dosage at the proteome level. Whether similar compensatory mechanisms operate in *Candida albicans* and how quickly they emerge remains unknown. Here, we leveraged a collection of over 200 natural *Candida albicans* strains isolated from diverse clinical and environmental sources, each carrying whole-chromosome aneuploidies, to study the molecular evolution of aneuploidy adaptation. First, we tracked the fitness and chromosomal stability of these isolates under non-stress conditions over 150 generations. Surprisingly, common aneuploidies (e.g., Chr4) tend to be unstable, while rarer ones (e.g., Chr6, Chr7) often persist, suggesting differential cellular constraints. We are now evolving 40 stable aneuploid and 40 euploid isolates under drug-exposed and drug-free conditions whilst collecting longitudinal proteomic data. Using this data, we will a) define common patterns of aneuploidy acquisition or loss; b) compare global proteome profiles of aneuploid strains under drug- and drug-free conditions; and c) assess the development and extent of protein-level dosage compensation. We aim to identify and interfere with key processes underlying aneuploidy adaptation, ultimately providing novel strategies to improve the outcomes for patients with fungal infections.

BLOCK B

Rapid Screening of Antifungal Drug Tolerance and Resistance Mechanisms in *C. albicans* using Transposon Mutagenesis

Sofya Rabinovich[1], Dr. Austin Mottola[1,2], Prof. Judith Berman[1]

[1]Tel Aviv University, Israel. [2]Berlin Institute of Health (BIH) at Charité, Germany

Abstract

Candida albicans is an opportunistic fungal pathogen. Antifungal treatment failures occur more frequently (~30%) than predicted by drug resistance prevalence (<3%). Antifungal tolerance - due to slow growth of a subpopulation of cells at supra-MIC drug concentrations - may explain these discrepancies: *C. albicans* strains exhibit a broad range of tolerance levels, with 31% having considerable tolerance levels. Tolerance depends upon diverse stress response pathways as well as genes that affect components and enzymes involved in membrane and cell wall integrity. To identify genetic determinants of fluconazole (FLC) tolerance, we performed a genetic screen using a genome-wide pooled *in vivo* transposon mutagenesis using an AcDs-derived transposon (1). The system leverages a haploid *C. albicans* strain and Illumina sequencing to identify mutants that are over- or under-represented following drug exposure. Thus far, we have identified genes involved in lipid metabolism, cell wall integrity, and intracellular trafficking, with mutations that exhibit increased or decreased competitive fitness under FLC selection. We are now screening selected candidate genes to ask if the mutations are causal and if any causality holds across diverse genetic backgrounds in modulating drug susceptibility. The transposon-mutagenesis approach provides a relatively rapid identification of pathways affecting selective growth under specific stress, and identifies possible mechanisms by which the drug affects *C. albicans* adaptation to the relevant stress conditions.

1. Segal, E.S. *et al.* (2018) mBio. e02048-18. https://doi.org/10.1128/mBio.02048-18.

BLOCK B

Deep Learning based Identification of *Candida albicans* on Organoid-derived-monolayers for live-Imaging

Alexandra Löwe[1], Charlotte Schwister[1], Sebastian Reusch[1], Katharina Jahn[2], Volker Rickerts[1], Nils Körber[1]

[1]Robert Koch-Institute, Germany. [2]Free University Berlin, Germany

Abstract

The polymorphic nature and shared morphology of fungi complicates their identification and localization on many habitats, including mucosal surfaces hindering phylogeographic studies. To address this limitation, we used deep learning for label-free detection of *Candida albicans* cells in live microscopy.

We infected organoid-derived monolayers (ODMs) of respiratory tissue with *C. albicans* and monitored the infection dynamics over 72 hours. The Calcofluor staining of individual fungal cells was utilized to train a neural network. The resulting neural network can reliably identify fungal cells on ODMs from brightfield images without additional staining. We used the trained model to identify *C. albicans* cells from time-lapse images to quantify *Candida* growth rate. Furthermore, we observed formation of hyphae and host-cell invasion of *C. albicans*.

This approach has the potential to facilitate spatial mapping, extend to other fungal species, assess community responses to external stressors, and evaluate reactions to different mucus conditions. Potential use cases range from the investigation of specific diseases conditions such as cystic fibrosis to test the effects of anti-fungal substances on colonization and infection dynamics. The label-free automatic detection of pathogens combined with an organoid model offers novel ways to understand and investigate host-pathogen interactions.

BLOCK B

ORP9 governs repair of epithelial cell plasma membrane damage caused by Candida albicans

Quynh T. Phan[1], Jianfeng Lin[1], Scott G. Filler[1,2]

[1]Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA, USA. [2]David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Abstract

Epithelial cell damage is a pathogenic feature of oropharyngeal candidiasis. Oxysterol binding protein related protein 9 (ORP9) is a lipid transfer protein that has recently been found to regulate the repair of large wounds in the plasma membrane (PM). We investigated the role of OPR9 in governing the extent of PM damage in TR146 oral epithelial cells infected with *C. albicans*. By confocal microscopy, ORP9 was observed to accumulate around hyphae in the infected cells. To determine the role of ORP9 on the host response to *C. albicans*, we deleted ORP9 in TR146 cells. Although deletion of ORP9 had no effect on *C. albicans* adherence or invasion, it increased the extent of PM damage by 1.7-fold, as measured by propidium iodide staining. This increase in damage was accompanied by a 1.5-fold increase in epithelial cell IL-8 secretion. Notably, deletion of ORP9 had no effect on PM damage caused by a *C. albicans ece1*Δ/Δ mutant or candidalysin alone, indicating that ORP9 repairs PM damage that is not due to candidalysin alone. Deletion of ORP9 also reduced the accumulation of phosphatidylinositol-4 kinase type 2α (PI4K2A) and its product, phosphatidylinositol-4-phosphate (PI4P) around *C. albicans* hyphae. PI4P is known to be a key signaling molecule in PM repair. FM464 staining indicated that deletion of ORP9 caused a substantial increase in membrane blebbing around the hyphae, suggesting that the ESCRT III pathway may be activated as a compensatory response. Thus, ORP9 plays a central role in governing PM repair during *C. albicans* infection.

BLOCK B

UNRAVELLING THE MOLECULAR MECHANISMS OF HOST HEME TRANSFER BY CFEM HEMOPHORES IN Candida albicans

Soham Chattopadhyay, Elian Far, Sunanda Dey, Udita Roy, Daniel Kornitzer

Department of Molecular Microbiology, B. Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa 31096, Israel, Israel

Abstract

Candida albicans is a prominent opportunistic fungal pathogen adept at harnessing host-derived heme to satisfy its critical iron requirements. Central to its acquisition mechanism is the family of CFEM (Common in Fungal Extracellular Membrane) hemophore proteins, notably Rbt5, Pga7, and Csa2, which extract heme from host proteins and enable its transport across the fungal cell envelope. Despite structural and biochemical knowledge regarding these proteins, the precise molecular mechanisms underlying their interactions and heme trafficking dynamics are not known. In this study, we harnessed fluorescence quenching by heme binding to fluorescent protein (FP)-tagged CFEM hemophores produced recombinantly in *Pichia pastoris*, to dissect the kinetics of heme transfer and infer the reaction mechanisms. Kinetic analyses revealed predominantly first-order reaction dynamics in heme transfer from host hemoglobin, indicative of a stochastic rather than direct protein-protein dependent release mechanism. Conversely, analysis of inter-hemophore heme transfer revealed distinctly second-order kinetics, strongly suggesting significant protein-protein interactions. Moreover, targeted mutagenesis of a predicted Csa2 dimerization site significantly altered reaction kinetics, reducing heme transfer efficiency and shifting the kinetics towards first-order, implicating the dimerization interface as critical for CFEM protein function. Collectively, our findings offer greater mechanistic clarity into fungal heme uptake pathways and illuminate potential molecular targets for innovative antifungal therapies.

BLOCK B

Short-term azole exposure can enhance *Nakaseomyces glabratus* pathogenesis

Gabriela Ribeiro[1,2], Weronika Danecka[3], Logan Tomlinson[2], Edward Wallace[3], Delma Childers[2]

[1]University of Guelph, Canada. [2]Aberdeen Fungal Group, University of Aberdeen, United Kingdom. [3]University of Edinburgh, United Kingdom

Abstract

Antifungal drug resistance and related phenomena in fungal pathogens, including tolerance, persistence and heteroresistance, have concerning implications for clinical treatment failure. This concern has led to a growing body of work to understand the molecular mechanisms driving responses to antifungals over extended timescales. Azole antifungal drugs directly inhibit lanosterol 14-α-demethylase, and indirectly affect the expression of carbohydrate metabolism, transmembrane transporters, and cell wall organization genes. It is not known how these indirect azole effects depend on dose, timing, and specific azole used, or how they influence host interactions. *Nakaseomyces glabratus* (formerly *Candida glabrata*) is the second leading cause of candidiasis, and clinical strains have high rates of intrinsic resistance to azoles. We investigated the early responses of reference strains BG2 and CBS138 to sub-inhibitory doses of fluconazole and voriconazole, and particularly, how these responses affect host-pathogen interactions. Transcriptomic and cell wall profiling data revealed highly similar responses for each strain to both azoles, including the upregulation of several virulence factors, such as adhesins and yapsins. We also observed significant increases in CBS138 survival in macrophages and hypervirulence in *Galleria mellonella* after voriconazole exposure. Using a combination of pharmacological inhibitors and deletion strains, we determined that voriconazole-enhanced virulence requires the yapsin protease *YPS1* and is regulated via the calcineurin pathway and the PKC cell wall integrity pathway, both of which regulate *YPS1* expression. Our study provides new insight into short-term azole adaptation in *N. glabratus*, and importantly demonstrates that sub-inhibitory azole exposure can induce virulence factors and enhance fungal pathogenesis.

BLOCK B

The Gcn5 lysine acetyl transferase mediates antifungal drug resistance and skin colonization of Candida auris

Manju Chauhan, Raju Shivarathri, Jenish Patel, Jigar Desai, Neeraj Chauhan

Hackensack Meridan Health, Center for Discovery and Innovation, USA

Abstract

Candida auris is a multidrug-resistant fungal pathogen that has a unique ability to grow and persist on human skin. However, the molecular mechanisms underlying pronounced multidrug resistance and host–pathogen interactions are poorly understood. Therefore, the objective of this study is to better understand and dissect the molecular and genetic basis of antifungal drug resistance as well as the skin colonization of *C. auris*.

GCN5 encodes a lysine acetyltransferase and functions as a coactivator in transcriptional regulation to modulate cell development and differentiation. We report here that *C. auris* Gcn5 lysine acetyltransferase is essential for echinocandin resistance. By genetic analysis, we have discovered that the function of Gcn5 is conserved across all five clades of *C. auris*. Notably, transcriptomics studies have identified sphingolipid metabolism and glycosylphosphatidylinositol (GPI) anchor biosynthetic pathways to be affected in echinocandin resistant *C. auris* strains in a *GCN5* dependent manner, indicating their potential roles in echinocandin resistance. Additionally, we have uncovered that Gcn5 masks the exposure of immunostimulatory cell-wall β-glucans, which inhibits the fungal phagocytosis and clearance by the myeloid phagocytes. Consequently, *gcn5Δ* mutant is severely impaired in dermal persistence and systemic virulence, assessed using two distinct murine models of skin colonization and systemic infection. These data indicate that Gcn5 is a central regulator of echinocandin and anti-fungal host resistance.

BLOCK B

Identifying putative hyphal regulators of *Candida albicans*

Dakota Archambault[1,2], Michael Lorenz[1,2]

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

Abstract

How *Candida albicans* interacts with phagocytes is an important determinant for the development of infection. Its interaction with host macrophages is complex with hyphal growth as a key feature that contributes to macrophage damage and escape from phagocytosis. Transcriptomic data from several *Candida* species post-phagocytosis identified two sets of uncharacterized genes as candidate virulence factors. The first set is strongly induced in phagocytosed *C. albicans* cells, and 48% of these have no known function. We generated a library consisting of deletion mutants in each of these 86 genes. Through our screening approach, we identified one hypofilamentous mutant strain in the gene *C2_04280W*, which has hyphal defects phenotypes on solid agar, invasion into an agar surface, adherence to a solid surface, inability to escape a macrophage and decreased virulence in our *in vivo C. elegans* model. Further research for this gene will focus on investigating the function of *C2_04280W* as a putative kinase and investigating virulence defects *in vivo*. The second set of genes are more highly expressed in phagocytosed *C. albicans* cells compared to other less virulent species. This set is highly enriched for hyphal-associated genes and includes six uncharacterized genes. These six genes include orthologs of an amino acid transporter, a deamidase, and a homolog of a morphology regulator from *S. cerevisiae*. We have generated deletion mutants of these six uncharacterized genes and are testing these deletions strains for impacts on hyphal growth.

BLOCK B

Mechanistic Insights into a Small Molecule Inhibitor of *Candida albicans* Filamentation

Kendall Esparrago, Paola Zucchi, José Lopez-Ribot, Jesús Romo

University of Texas at San Antonio, USA

Abstract

Background: *Candida albicans* is a critical priority pathogen. Its ability to transition from yeast to hyphae is essential for pathogenesis and represents an attractive therapeutic target.

Goals/Hypothesis: We previously identified compound 9029936, a small molecule that inhibits filamentation and biofilm formation *in vitro* and in murine models of disseminated, oropharyngeal candidiasis, and in a catheter model. We aim to determine the molecular target(s) of this small molecule and hypothesize that it targets key regulators downstream of *BRG1*, a master regulator of filamentation.

Methods: We assessed the effects of 9029936 on *C. albicans*, *C. dubliniensis*, *C. lusitaniae*, and *C. parapsilosis* by measuring growth and performing cell wall staining (Calcofluor White and Wheat Germ Agglutinin). We serially passaged *C. albicans* for 40 days in the presence of the compound. Resistant strains were analyzed for growth and biofilm formation.

Results: The compound induced dose dependent growth defects in *Candida* species, indicating inhibition of filamentation at low concentrations and general growth at higher concentrations. Cell wall staining revealed increased exposed chitin, suggesting altered cell wall architecture. No effects were observed in *Nakaseomyces glabratus* or *Saccharomyces cerevisiae*, suggesting species-specific targeting. Lastly, resistant *C. albicans* mutants emerged after serial passage capable of filamenting under all tested conditions.

Future Directions: We are screening the GRACE library and applying click chemistry to identify the molecular target(s). Whole-genome sequencing of resistant strains will further refine our search.

Conclusions: This work may uncover novel regulators of filamentation and lay the groundwork for new antifungal therapies targeting virulence.

BLOCK B

Identification of novel plasma membrane eisosome proteins in *Candida albicans* by TurboID proximity labeling

Carla Lanze, John Haley, James Konopka

Stony Brook University, USA

Abstract

Candida albicans virulence is promoted by the ability of this human fungal pathogen to resist stressful conditions in the host and to undergo invasive hyphal growth into tissues. Specialized plasma membrane subdomains that have been termed Membrane Compartment of Can1 (MCC), also known as eisosomes, are important for these processes. MCC/eisosome domains are furrow-shaped invaginations of the plasma membrane that are about 250 nm long and 50 nm deep. To identify novel proteins that reside in eisosomes, the TurboID variation of the BioID proximity labeling method was optimized for use in *C. albicans*. This method employs a mutant form of the BirA biotin ligase (TurboID) that results in biotinylation of nearby proteins, facilitating their purification and identification by mass spectrometry. TurboID was fused to two proteins, Sur7 and Lsp1, that localize to eisosomes and are important for virulence. The results identified 12 known eisosome proteins as well as a large set of potential new eisosome proteins. Analysis of 19 candidate proteins by Green Fluorescent Protein (GFP) tagging identified 7 new proteins that co-localized with eisosomes. One of the new eisosome proteins, Ker1, was important for invasive growth into agar medium and resistance to stress caused by copper and cell wall perturbing agents. Previous studies showed that Ker1 is important for virulence (Galan et al., 2004. PMID: 15289560). Thus, TurboID succeeded in identifying novel eisosome proteins and can be applied to aid in defining the mechanisms of other aspects of *C. albicans* pathogenesis.

BLOCK B

Evolution of altered iron acquisition in *Candida spp.* in chronic cystic fibrosis (CF) infections

Kiran Mirpuri, Daniel Murante, Deborah Hogan

Dartmouth College, USA

Abstract

Mucus accumulation in the airways of people with cystic fibrosis (CF) affects pathogen clearance and causes the establishment of microbial infections which frequently include fungi leading to chronic inflammation in the lung. In the inflammatory lung environment, mutations repeatedly arise that likely increase fitness in the airway environment. In *C. albicans*, mutations in gene encoding the Nrg1 transcriptional repressor are observed and in *C. lusitaniae* and *Exophilia dermatiditis* mutations in *MRS4* are common. In addition, within the gastrointestinal tract and lungs of select patients with CF, we have found isolates with *nrg1* mutations. Several lines of evidence suggest that both the loss of *nrg1* in *C. albicans* and *mrs4* in *C. lusitaniae* lead to the upregulation of genes involved in iron acquisition. One gene in the iron acquisition regulon, *CFL2*, a putative ferric reductase, has strong oxidation properties that might contribute to microbe-host or microbe-microbe interactions. These results suggest that iron limitation, driven through nutritional immunity, is a key selective pressure to fungal adaptations to the CF lung. Furthermore, these iron restriction mutants may cause more damage. Future studies will allow us to understand how the metal limiting environment of the CF lung may aid in its competition against other pathogens or the host immune system.

BLOCK B

Linking Candida albicans protein kinases to cytotoxity – characterisation of Crk1 functions during epithelial invasion

Anna Möslinger[1], Bernardo Ramírez-Zavala[2], Tim B. Schille[1,3], Joachim Morschhäuser[2], Lydia Kasper[1], Stefanie Allert[1], Mark S. Gresnigt[4], Bernhard Hube[1,3,5]

[1]Department of Microbial Pathogenicity Mechanisms, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Germany. [2]Institute of Molecular Infection Biology, University of Würzburg, Germany. [3]Cluster of Excellence Balance of the Microverse, Friedrich Schiller University Jena, Germany. [4]Junior Research Group Adaptive Pathogenicity Strategies, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Germany. [5]Institute of Microbiology, Faculty of Biological Sciences, Friedrich Schiller University, Germany

Abstract

Candida albicans exists as a commensal and pathogen depending on environmental conditions. Both stages require a complex network of signalling cascades, which are mostly regulated by protein kinases. The *C. albicans* genome was predicted to encode 108 protein kinases yet nearly 50% remain uncharacterised. In this study, we dissect the role of *C. albicans* protein kinases during the transition from commensalism to pathogenicity.

We screened a library containing individual *C. albicans* mutants lacking each of the identified protein kinase genes for their cytotoxicity against intestinal epithelial cells (IEC) *in vitro*. Mutants displaying altered IEC cytotoxicity were further characterised for their growth ability and morphology phenotypes. Surprisingly, *CRK1* deletion caused increased IEC cytotoxicity compared to the parental wild-type, despite slower growth and reduced hyphal length. Infection of various epithelial cell lines in different cell culture media revealed that the increased damage potential of *crk1*Δ/Δ is cell type- and media-dependent. Accordingly, metabolic profiling and spot dilution assays showed that *CRK1* is important for metabolic adaptations and resistance to cell wall stress. When investigating the stages of IEC infection, we observed slightly reduced adhesion for *crk1*Δ/Δ, whereas invasion was significantly increased in line with the increased cytotoxic potential. In contrast, translocation of *crk1*Δ/Δ through an IEC barrier in a transwell assay was reduced.

Collectively, we identified the protein kinase Crk1 as a critical regulator of processes preventing excessive intestinal epithelial cell cytotoxicity.

BLOCK B

The hyphal morphology shapes antifungal immunity

Axel Dietschmann[1], Laszlo Musiejovsky[2], Thomas Krüger[3], Collins Boahen[4], Sude Dinçer[1], Fay Bruggeman[1], Ceren Oktay[1], Maximilian Himmel[1], Stefan Wirtz[5], Eicke Latz[6], Jürgen Ruland[7], Olaf Kniemeyer[3], Frank van de Veerdonk[8], Vinod Kumar[4], Gernot Schabbauer[2], Mark Gresnigt[1]

[1]Junior Research Group Adaptive Pathogenicity Strategies, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Jena, Germany. [2]Institute for Vascular Biology, Centre for Physiology and Pharmacology, Medical University Vienna, Vienna, Austria; Christian Doppler Laboratory for Arginine Metabolism in Rheumatoid Arthritis and Multiple Sclerosis, Vienna, Austria. [3]Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Jena, Germany. [4]Department of Internal Medicine and Radboud Institute of Molecular Life Sciences (RIMLS), Radboud University Medical Center, Nijmegen, Netherlands. [5]Deutsches Zentrum für Immuntherapie (DZI), Friedrich-Alexander University Erlangen-Nürnberg (FAU) and Universitätsklinikum Erlangen, Germany; Department of Internal Medicine 1, Friedrich-Alexander University Erlangen-Nürnberg (FAU) and Universitätsklinikum Erlangen, Erlangen, Germany. [6]Institute of Innate Immunity, University Hospital, University of Bonn, Bonn, Germany. [7]Institute of Clinical Chemistry and Pathobiochemistry, School of Medicine and Health, Center for Translational Cancer Research (TranslaTUM), Munich, Germany; German Cancer Consortium (DKTK), partner site Munich, Germany; German Center for Infection Research (DZIF), partner site Munich, Germany. [8]Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, Netherlands

Abstract

Filamentation is a common virulence attribute, applied by many fungal pathogens to invade tissues and infect susceptible hosts. Particularly in *Candida albicans*, filamentation is linked to virulence factor expression, rendering hyphae a primary threat, which tissue-resident macrophage sentinels have to disarm in early stages of infection. This is often neglected in conventional *in vitro* models, leaving it unresolved, how the initial confrontation between human macrophages and *C. albicans* hyphae would differentially steer downstream immunity, when compared to yeast cells. Interestingly, hyphae more than yeasts incited primary human macrophages to create an environment that potently activates neutrophils, key recruited antifungal effector cells. To understand, how macrophages communicate morphological distinctions, we analyzed the macrophage exoproteome and visualized the interaction of macrophages with fungal morphologies by live-cell imaging. Thereby, we discovered that neutrophil activation was associated with prior hyphal triggering of host-regulated, macrophage-specific, inflammatory cell death, which neither resembled pyroptosis nor necroptosis. Instead, specific antifungal recognition receptors like Dectin-1, Mincle and CD11b and downstream FAK, PI3K and ROS crucially contributed to “hyphoptosis”. This cell death was measurable *ex* and *in vivo*, where it drives neutrophilic inflammation. Further, candidemia

patients exhibit different allelic distribution within “hyphoptosis”-relevant genes and macrophages of primary immunodeficiency patients display elevated susceptibility to engage “hyphoptosis”. Collectively, we propose a novel, hyphal triggered, regulated inflammatory cell death pathway, which if dysregulated may become a major risk factor for candidiasis. Currently, we aim to harness our understanding of “hyphoptosis” to advance immunotherapeutic approaches against fungal infections.

BLOCK B

OMICS identification of azole-resistance genes in a "non-clinical" *C. glabrata* strain

Maria Joana Pinheiro[1,2], Ana Mendes-Ferreira[3,4], Kyle W Cunningham[5], Nuno Pereira Mira[1,2]

[1]iBB, Institute for Bioengineering and Biosciences, Instituto Superior Técnico – Department of Bioengineering, Universidade de Lisboa, Lisbon, Portugal., Portugal. [2]Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal., Portugal. [3]WM&B – Laboratory of Wine Microbiology & Biotechnology, Department of Biology and Environment, University of Trás-os-Montes and Alto Douro, 5001-801, Vila Real, Portugal, Portugal. [4]BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016, Lisbon, Portugal, Portugal. [5]Department of Biology, Johns Hopkins University, Baltimore, Maryland, USA, USA

Abstract

Candida glabrata (now *Nakaseomyces glabratus*) is a human pathobiont commonly found as a commensal in the gastrointestinal and genitourinary tracts, capable of causing severe disseminated infections. Beyond its clinical relevance, *C. glabrata* has also been isolated from diverse non-clinical sources, including soil, plant surfaces (such as fruits and leaves), decaying wood, tree bark, and marine-associated environments such as coastal waters and beach sand. Azoles are the frontline therapy for treating infections caused by *C. glabrata*, and understanding resistance mechanisms is essential to improving treatment and diagnosis. While azole resistance has been extensively studied in laboratory strains and, to a lesser extent, in clinical isolates, little is known about these mechanisms in environmental strains.

In this study, we addressed this gap by investigating azole resistance in an environmental strain, UTAD68, isolated from grape must. Our aim was to understand how the distinct evolutionary history of this strain might have shaped its repertoire of azole resistance genes. Karyotyping revealed notable genomic differences between UTAD68 and clinical strains, including chromosomal rearrangements (e.g., translocations on chromosomes I and L), as well as substantial divergence in alleles related to adhesion, transport, and transcriptional regulation. Results from in vitro adaptive evolution experiments and Hermes transposon-based mutagenesis identified genes and genetic alterations associated with azole resistance in UTAD68. These findings were compared with data from clinical strains, revealing both shared features and significant differences, highlighting the critical influence of genetic background on the mechanisms underlying azole resistance.

BLOCK B

A *Candida auris* patient's strain harbors a circular chromosome 3 which duplication leads to markedly distinct expression profile and phenotypes

Hassan BADRANE[1], Shaoji Cheng[1], Adam Glawe[2], Jeniel Nett[2], Christopher Dupont[3], M. Hong Nguyen[1], Cornelius J. Clancy[1,4]

[1]University of Pittsburgh School of Medicine, USA. [2]University of Wisconsin-Madison, USA. [3]J. Craig Venter Institute, USA. [4]V.A. Pittsburgh Healthcare System, USA

Abstract

Since *Candida auris* emerged in Japan in 2009, it spread to all continents. The first U.S. cases came in 2016 as outbreaks of 51 cases in 4 states and exponentially increased to reach 4,514 cases in 2023. *C. auris* displayed a great diversity with 6 genetically distinct clades. In 2021, we collected 41 randomly-chosen clones from a patient's blood culture bottle. After whole genome sequencing and an almost telomere-to-telomere assembly, we found they harbored 7 linear chromosomes, except for chromosome 3 which surprisingly was circular (cirChr3). Assuming this cirChr3 originated from a linear version, it appears that it was fixed in the population, meaning it conferred an advantage at some point after the circularization. In addition, 6/41 clones presented a duplication of cirChr3 among which 1 clone had a partial duplication. The duplication was confirmed using qPCR with a set of 4 genes dispersed on Chr3. When compared to clones with 1x cirChr3, those with duplicated cirChr3 had a slightly delayed growth, displayed less adhesion to plastic, grew less biofilm on plastic and skin, and lost the aggregator phenotype. Using RNA-seq, they were found to have a markedly distinct expression profile. There were no differences in antifungal resistance profiles. Passage of 2 clones on YPD showed the cirChr3 was maintained after ~ 120 generations, which implies it is stable through mitosis. In conclusion, the rise and fixation of a stable cirChr3 in *C. auris* led to the advent of clones with a duplication leading to markedly distinct expression profile and phenotypes.

BLOCK B

Structure-guided optimization of small molecules targeting the casein kianseYck2 as a strategy to combat drug-resistant *Candida albicans*

Bonnie Yiu[1], Meganathan Nandakumar,[2], Peter J Stogios[1], Emily Puumala[1], Benjamin G Strickland[2], Robert Zarnowski[3], Xiaoyu Wang[4], Noelle S Williams[4], Alexei Savchenko[1,5], David R Andes[3], Luke Whitesell[1], Timothy M Willson[2], Nicole Robbins[1], Leah E Cowen[1]

[1]University of Toronto, Canada. [2]University of North Carolina at Chapel Hill, USA. [3]University of Wisconsin-Madison, USA. [4]University of Texas Southwestern Medical School, USA. [5]University of Calgary, Canada

Abstract

Candida albicans is a common cause of life-threatening fungal infection in the developed world but remains a therapeutic challenge. Treatment of systemic fungal infections is limited to four front-line antifungal classes, including the echinocandins, which function by inhibiting cell wall synthesis. Protein kinases have been rewarding drug targets across diverse indications but remain untapped for antifungal development. Previously, screening kinase inhibitors against *C. albicans* revealed a 2,3-aryl-pyrazolopyridine, GW461484A (GW), which targets casein kinase 1 (CK1) family member Yck2. Yck2 is required for growth under physiological conditions, is important for echinocandin resistance, and plays a role in virulence in a mouse model of infection, however the mechanism by which is regulates these traits remains enigmatic. Moreover, GW suffers from poor metabolic stability and thus presents a liability for its progression into *in vivo* studies. Here, we report optimization of GW via two complementary approaches, synthesis of bioisosteres possessing an imidazo[1,2-a]pyridine core, and R-group substitution of GW's pyrazolo[1,5-a]pyridine core. Characterization of compounds revealed multiple derivatives with improved pharmacological properties that retain whole-cell bioactivity and selectivity for fungal Yck2 compared to human CK1α or p38α. Efficacy studies in mice identified analogs with single-agent and echinocandin potentiating activities against a *C. albicans* echinocandin-resistant isolate. Finally, phosphoproteomic analysis identified multiple downstream Yck2 effectors that govern echinocandin-induced stress. Overall, these results validate Yck2 as an antifungal target, identify novel circuitry through which casein kinases regulate echinocandin susceptibility, and encourage further development of inhibitors acting by this previously unexploited mode of action.

BLOCK B

Telomere-to-telomere Phased Diploid Assembly of the Candida albicans SC5314 Reference Genome.

Darren Abbey[1], Christopher Zajac[1], Wendy Phillips[1], Ursula Oggenfuss[1], Alessia Buscaino[2], Matt Anderson[3], Lois Hoyer[4], Anna Selmecki[1]

[1]University of Minnesota, USA. [2]University of Kent, United Kingdom. [3]University of Wisconsin, USA. [4]University of Illinois, USA

Abstract

Since its initial release in 2004, the Candida albicans SC5314 reference genome has been a foundational resource for studying the biology and pathogenicity of this major fungal pathogen. However, current assemblies lack telomere-to-telomere continuity and diploid phasing, limiting their utility for resolving structural variants, allele-specific expression, and genome dynamics critical to understanding C. albicans adaptation. To address these limitations, we generated an improved SC5314 reference genome using long-read sequencing technologies (PacBio and Oxford Nanopore) across five independent runs from different laboratories. This telomere-to-telomere phased diploid assembly corrects errors in previous versions and reveals numerous structural variants between homologous chromosomes. To better characterize complex genomic regions, we developed a novel pipeline to analyze repetitiveness across the genome. This enabled detailed resolution of challenging regions such as rDNA repeats and Major Repeat Sequence (MRS) loci. We identified a previously unreported MRS locus on Chromosome 4 and observed significant sequence divergence among MRS loci, suggesting limited recombination between them. We also mapped transposable elements (TEs) genome-wide, identifying haplotype-specific insertions and a novel TE type. At the nucleotide level, our assembly resolves all 3,559 ambiguous bases and unresolved regions (Ns) from prior assemblies, including those within 499 open reading frames (ORFs). Genome annotation further revealed ~20 new gene paralogs. The updated reference genome and associated computational tools will be made publicly available via the Candida Genome Database and the Yeast Analysis Mapping Pipeline, providing a valuable resource to advance both clinical and laboratory research on C. albicans.

BLOCK B

Elevated vaginal heparan sulfate correlates with impaired neutrophil killing of *Candida albicans* in women with vulvovaginal candidiasis

Junko Yano[1], Nicole Woznicki[2], Jack Sobel[3], Mary Meyaski-Schluter[4], Paul Fidel[1]

[1]Department of Oral and Craniofacial Biology, School of Dentistry, LSU Health New Orleans, Louisiana, USA. [2]Department of Obstetrics and Gynecology, Wayne State University, Detroit, Michigan, USA. [3]Division of Infectious Diseases, Wayne State University, Detroit, Michigan, USA. [4]Clinical and Translational Research Center, LSU Health New Orleans, Louisiana, USA

Abstract

Recurrent vulvovaginal candidiasis (RVVC), primarily caused by *Candida albicans*, is a common infection affecting a significant number of women worldwide. Despite a robust inflammatory response with high numbers of polymorphonuclear neutrophils (PMNs) during symptomatic episodes, fungal clearance often fails, leading to persistent fungal growth and PMN-driven immunopathology. Studies in an established mouse model revealed that vaginal heparan sulfate (HS) acts as a competitive inhibitor for PMN-*C. albicans* interactions, impairing fungal clearance despite otherwise normal PMN function. This study investigated the presence and inhibitory role of HS in women diagnosed with RVVC. Design included use of vaginal conditioned medium (VCM) comprising vaginal secretions collected via broad-tip foam swabs from women with symptomatic RVVC, those in asymptomatic remission, and healthy controls. Results from ELISA and immunostaining showed significantly elevated HS levels in VCM and vaginal epithelial cells from symptomatic women compared to asymptomatic and healthy controls. PMN killing assays revealed significantly reduced antifungal activity in the presence of VCM from symptomatic women compared to asymptomatic and control VCM, with a significant negative correlation between vaginal HS concentrations and PMN antifungal activity. The inhibitory effect of HS was further confirmed by impaired PMN killing in control VCM spiked with purified HS, and by the restoration of antifungal activity following heparanase (HS lyase) treatment of both symptomatic VCM and HS-spiked control VCM. These findings support results from the animal model and provide clinical evidence that elevated HS in the vaginal environment contributes to PMN dysfunction, leading to persistent *C. albicans* overgrowth and VVC-associated immunopathology.

BLOCK B

Yapsins from *Candida glabrata* (*Nakaseomyces glabratus*) affect host barrier integrity, immune responses, and antifungal susceptibility

Dorota Satala, Mikolaj Harasimowicz, Ewelina Wronowska, Aneta Bednarek, Kamil Maslanka, Kamila Kulig, Maria Rapala-Kozik

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

Abstract

Candida glabrata (currently classified as *Nakaseomyces glabratus*) unlike *Candida albicans*, which secretes a well-characterized family of aspartic proteases (Saps), expresses a distinct family of cell surface-associated aspartic proteases known as yapsins (Yps). Although previous studies have suggested the involvement of yapsins in fungal homeostasis, their precise role in host-pathogen interactions remains poorly defined. Here, we investigated the dynamics of Yps binding and its impact on barrier integrity in A431 and Cal-27 epithelial cell lines, using *YPS* gene deletion mutants and fluorescently or biotin-labeled proteases. In parallel, we assessed their immunomodulatory effects in THP-1 monocytes and in the invertebrate *Galleria mellonella* infection model. Both Yps3 and Yps9, purified from the secretome, showed marked binding to epithelial cell surfaces and disrupted the integrity of the epithelial monolayer. However, deletion of *YPS* genes only caused minor alterations in fungal adhesion. Assessment of inflammatory responses in THP-1 monocytes revealed a slight modulation of IL-1β and IL-8 secretion after exposure to purified yapsins. While pretreatment of *G. mellonella* larvae with Yps3 and Yps9 clearly increased larval survival, highlighting their potential role in immune priming. Finally, a preliminary assessment of antifungal susceptibility indicated that deletion of *YPS* genes might influence fungal survival in the presence of caspofungin and amphotericin B. Overall, these results underscore the complex and multifunctional nature of Yps proteins in host interactions, epithelial barrier disruption, and immune modulation, justifying their further exploration as potential therapeutic targets against *C. glabrata* infections.

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BLOCK B

A natural *Candida albicans* - *Candida glabrata* co-isolate exhibits synergistic growth and increased virulence in host model

Rahma Ute[1], Margy Alejandra Esparza Mora[1], Celia Funes[1], Judith Berman[2], Markus Ralser[1,3]

[1]Charité Universitätsmedizin Berlin, Germany. [2]Tel Aviv University, Israel. [3]Nuffield Department of Medicine, University of Oxford, United Kingdom

Abstract

Infections rarely come alone. The ability of a pathogen to cause disease is shaped by its surrounding environment. *Candida albicans* is a well-known human commensal and opportunistic pathogen that can cause infection in immunocompromised individuals. While extensively studied as a single organism, the interactions between *C. albicans* and other fungal species is not well understood. From a comprehensive library of what was thought to only contain natural *C. albicans* isolates, we identified 20 coisolates, the majority of which were *C. albicans–Candida glabrata* pairings. We hypothesized that fungal-fungal interactions within these coisolates might enhance growth and virulence compared to each species grown independently.

To test this, we first screened the growth of the 20 coisolates and their respective species, selecting one coisolate with a distinct growth phenotype for further study. In vitro, a microbroth dilution assay using the antifungal fluconazole revealed that the coisolate exhibited enhanced growth in the concentration range of 64–8 μg/mL, supporting a synergistic interaction. In vivo experiments using *Galleria mellonella* demonstrated the coisolate had higher virulence than *C. albicans* alone.

These findings suggest a synergistic relationship between the species within the coisolate, potentially enhancing both growth and virulence. To further investigate the underlying mechanisms, we plan to explore this through proteomic analysis. The preliminary results highlight the importance of understanding fungal-fungal interactions in shaping pathogenic behaviors, resistance phenotypes and their role in drug tolerance.

BLOCK B

Immunomodulatory Effects of *Candida albicans* and *Candida parapsilosis* on Human Macrophage Polarization

Florabelle Cabarrubias[1,2], Anna Luelly Machado[1,2], Attila Gácser[1,2,3,4]

[1]Department of Biotechnology and Microbiology, University of Szeged, Szeged, Hungary. [2]HCEMM-USZ, Fungal Pathogens Research Group, Szeged, Hungary. [3]HUN-REN-SZTE Pathomechanisms of Fungal Infections Research Group, University of Szeged, Szeged, Hungary. [4]Competence Centre for Molecular Biology, Bionics, and Biotechnology, University of Szeged, Szeged, Hungary

Abstract

Candida species, including *Candida albicans* and *Candida parapsilosis*, are responsible for most systemic yeast infections in nosocomial settings. During invasion, these species interact with monocytes, influencing macrophage polarization. Macrophages can polarize into pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes in response to microenvironmental signals. We aimed to evaluate macrophage polarization upon *Candida* co-incubation.

Peripheral blood mononuclear cell-derived monocytes (PBMC-DMs) and M1-like macrophages were co-incubated with *C. albicans* (SC5314) and *C. parapsilosis* (CLIB 214). Macrophages were immunolabeled with CD68 (a macrophage marker), CD86 (an M1 marker), and CD163 (an M2 marker) and analyzed using flow cytometry. Cytokine levels of TNF-α, IL-6, TGF-β, and IL-10 were measured.

Results showed the M1/M2 ratio was unchanged by *C. albicans* exposure, similar to untreated controls. In contrast, PBMC-DMs co-incubated with *C. parapsilosis* had a significant M2 increase, suggesting a tolerogenic effect. M1-like macrophages exposed to *C. parapsilosis* exhibited a mixed M1+/M2+ population, indicating a transitional state.

Cytokine analysis further highlighted differences. PBMC-DMs exposed to *C. parapsilosis* showed elevated IL-6 and IL-10, while M1-like macrophages had increased TNF-α, IL-6, and IL-10. These findings suggest *C. parapsilosis* promotes an M2 phenotype in PBMC-DMs but induces a transitional M1/M2 state in M1-like macrophages.

Our study demonstrates that *Candida* species elicit distinct macrophage polarization responses during early fungal-macrophage interactions. These differences reflect the unique pathogenic strategies employed by *Candida* species and provide insights into developing targeted therapeutic strategies to combat *Candida* infections.

BLOCK B

Fungal extracellular vesicles potentially promote epithelial-mesenchymal transition in oral squamous cell carcinoma

Éva Veres[1], Dóra Izabella Adamecz[1,2], Zóra Szilovics[1], Gergő Svorenj[1], Krisztina Buzás[3], Mónika Kiricsi[2], Attila Gácser[4,5,6]

[1]Department of Biotechnology and Microbiology, University of Szeged, Hungary. [2]Department of Biochemistry and Molecular Biology, University of Szeged, Hungary. [3]Synthetic and System Biology Unit, Hungarian Academy of Sciences, Biological Research Centre (BRC), Szeged, Hungary. [4]HCEMM--USZ Fungal Pathogen Research Group, Department of Microbiology, Szeged, Hungary. [5]HUN-REN--SZTE Pathomechanisms of Fungal Infections Research Group, University of Szeged, Hungary. [6]University of Szeged, IKIKK, Competence Centre for Molecular Biology, Bionics and Biotechnology, Hungary

Abstract

Presence of a tumor can disrupt the balance of normal human microbiota, leading to an increased susceptibility to infections such as oral *candidiasis*. Our previous research showed that *Candida albicans* accelerates oral squamous cell carcinoma (OSCC) progression in vitro and in vivo by activating tumor-promoting pathways, like increasing matrix metalloproteinase (MMP) activity and oncometabolite production. However, the specific factors behind these phenomena are yet to be discovered. The current study investigates the role of extracellular vesicles (EVs) from *Candida albicans* in OSCC progression, as we believe EVs are key mediators in paracrine communication and possibly influence host-pathogen interactions related to cancer development.

The isolated EVs from both the yeast and hyphal forms of *Candida albicans* were characterized by nanoparticle tracking analysis and transmission electron microscopy. The uptake of fungal EVs by human OSCC tumor cells (HSC-2) was analyzed through flow cytometry and confocal microscopy. For more in depth understanding, we investigated the impact of *Candida*-derived EVs on epithelial-mesenchymal transition (EMT) in HSC-2 cells, which is a critical step in the formation of cancer metastasis, assessing tumor cell migration, as well as MMP activity, cytokine production, and the expression of EMT-related genes.

The results indicate that tumor cells can internalize fungal EVs through an energy-dependent mechanism. The exposure to *Candida*-derived EVs significantly alters cell migration and morphology, increasing MMP activity and cytokine production in HSC-2 cells. These findings suggest that *Candida*-EVs play a crucial role in promoting EMT and facilitate the progression of OSCC, contributing to a more aggressive cancer phenotype.

BLOCK B

Is your extracellular vesicle sample clean? An improved extracellular vesicle isolation protocol for *Candida* species

Éva Veres[1], Dóra Adamecz[1], Zóra Szilovics[1], Gergő Svorenj[1], Edit Buzás[2], Marcio Rodrigues[3], Attila Gácser[4,5,6]

[1]Department of Biotechnology and Microbiology, University of Szeged, Hungary. [2]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary. [3]Instituto Carlos Chagas, Fundação Oswaldo Cruz, Curitiba, Brazil. [4]HCEMM--USZ Pathogen Fungi Research Group, University of Szeged, Hungary. [5]HUN-REN- SZTE Pathomechanisms of Fungal Infections Research Group, University of Szeged, Hungary. [6]University of Szeged, IKIKK, Competence Centre for Molecular Biology, Bionics and Biotechnology, Hungary

Abstract

The phenomenon that fungi are capable of releasing extracellular vesicles (EVs) has been known since 2007. EVs are lipid bilayer-coated particles containing proteins and nucleic acids and may play key roles in interspecies communication and host-pathogen interactions. However, investigating EV function requires highly purified samples. Although several isolation methods have been developed over the years, none can fully prevent the co-isolation of non-EV particles and contaminations. Therefore, we aimed to develop an improved fungal EV isolation protocol.

To assess purification efficiency, we isolated EVs from cultures of *Candida* species using ultracentrifugation, followed by purification via size exclusion chromatography. To compare the purification efficiency, we used chromatography columns with two different pore diameters. Samples were evaluated based on protein, sterol, lipid content, and particle number. We checked the contamination status of the samples using transmission electron microscopy, and analyzed the size distribution of the EVs using nanoparticle analysis. We also performed proteomic, lipidomic, and transcriptomic analyses on both purified and crude samples. Naturally, to get a more comprehensive picture not just on the quality of the different EV samples, but on their biological relevance as well, experiments shall be conducted regarding the cellular biological effects of each fungal EV isolates.

Following size exclusion chromatography, we observed a reduction in the macromolecular content of non-lysed EV samples, indicating that co-isolated particles had been successfully removed. Therefore, we recommend combining ultracentrifugation with size exclusion chromatography for studies focusing on fungal EV-related effects, as this approach highly improves sample purity and reliability.

BLOCK B

A lichen secondary metabolite exhibits antibiofilm activity against fungal pathogens

Martin Odabas[1], Katharina Kainz[1], Ingo Weinberger[1], Kerstin Schloffer[1], Sabrina Riedl[1,2], Marylene Krugler[3], Dagmar Zweytick[1,2], Joel Boustie[3], Frank Madeo[1,2,4], Didac Carmona-Gutierrez[1,2]

[1]Institute of Molecular Biosciences, NAWI Graz, University of Graz, Graz, Austria. [2]BioHealth Graz, Graz, Austria. [3]Rennes Institute of Chemical Sciences, Université de Rennes 1, France. [4]BioTechMed Graz, Graz 8010, Austria

Abstract

Lichens are symbiotic organisms composed of a fungal partner, typically an ascomycete, and a photosynthetic algal or cyanobacterial partner. They are well known for producing unique secondary metabolites, some of which have been shown to exhibit medically relevant bioactivities, including antimicrobial effects. With the increasing prevalence of fungal infections and the growing resistance to commonly used antimycotics, there is an urgent need for new antifungal agents. In this study, we screened a collection of lichen-derived metabolites for antifungal properties in two medically relevant fungal pathogens, *Candida albicans* and *Nakaseomyces glabrata*. Several compounds exhibited inhibitory effects against planktonic cells and/or biofilm formation in at least one of these species. Notably, two related compounds demonstrated the strongest activity against biofilms, structures that contribute significantly to antifungal resistance. Among them, LC38 was the most effective in disrupting pre-formed biofilms and preventing biofilm formation, key challenges in clinical mycology. Importantly, LC38 showed no detectable toxicity to human cells at the half-maximal inhibitory concentrations assessed. These findings highlight lichen-derived metabolites as promising candidates for new antifungal therapies targeting resistant and biofilm-associated fungal infections.

BLOCK B

Genomic surveillance of commensal and clinical *Candida albicans* isolates

Maria J Carvalho[1], Ana R Guimarães[1], Doménica Rivadeneira[1], Rafaela Beleza[1,2], Ana Poim[1], Inês Correia[1], Gabriela Moura[1], Ana R Bezerra[1]

[1]Institute of Biomedicine (iBiMED), Department of Medical Sciences, University of Aveiro, Portugal. [2]Department of Biology, University of Aveiro, Aveiro, Portugal, Portugal

Abstract

Candida albicans is a common human commensal and the leading cause of invasive fungal infections in developed countries, with mortality rates reaching 40% despite treatment. Data from Portugal remain limited. To support ongoing surveillance of antifungal resistance and genomic diversity, we analysed 165 *C. albicans* isolates from the iBiMED biobank (n=505 *Candida* spp.) using whole-genome sequencing (Illumina).

Isolates' antifungal susceptibility profiles to amphotericin B, anidulafungin (AND), micafungin (MYC), itraconazole (ITZ) and fluconazole (FLZ) were established according to the EUCAST guidelines. Most isolates were susceptible to the antifungals tested, with 38 isolates resistant to AND and/or MYC and 12 isolates resistant/intermediate to FLZ and/or ITZ.

Genomic variation was evident in the form of polymorphisms, copy number variations, and loss of heterozygosity. Multilocus sequence typing revealed 80 sequence types (ST), from which 44 were novel, indicating shared ST with worldwide distributed *C. albicans* isolates and ST only found in Portugal.

We did not find previously reported genomic variations associated with antifungal resistance in most resistant isolates of our collection. Novel mutations within the same genomic regions and features were identified, and supervised and unsupervised analyses are ongoing to uncover new associations with resistance that have not been described before.

This work highlights significant genomic diversity and uncovers potential new markers of antifungal resistance, reinforcing the importance of continued *Candida* genomic surveillance. The iBiMED biobank collection of *Candida* spp. isolates continues to expand warranting the genomic surveillance of these organisms not only in Portugal but also in a worldwide context.

BLOCK B

Identification and characterisation of echinocandin heteroresistance in key *Candida* species

Ben Caswall[1], Heidi Olbrys[1], Rachel Wake[1,2], Elaine Bignell[3], Tihana Bicanic[1,3]

[1]City St George's, University of London, United Kingdom. [2]University of the Witwatersrand, South Africa. [3]University of Exeter, United Kingdom

Abstract

Antifungal heteroresistance is defined by a subpopulation existing within a clonal isolate, which displays reduced susceptibility to a particular drug, compared to the main population of cells. These subpopuluations are often undetected by conventional MIC testing, as they can be as small as 0.001% of the population. Echinocandin heteroresistance has been associated with breakthrough *Candida parapsilosis* infections following micafungin prophylaxis. Echinocandin heteroresistance has also been reported in one *Candidozyma auris* isolate so far. Mechanisms of resistance in echinocandin heteroresistant subpopulations have not been identified in any *Candida* species.

Echinocandin heteroresistance was assessed in 10 clinical isolates of 3 key pathogens; *Candida albicans, C. auris* and *Nakaseomyces glabratus.* Population analysis profiling was carried out to assess heteoresistance. Overnight cultures were plated onto increasing concentrations of anidulafungin-infused YPD agar. CFU counting was performed after 24h and 48h incubation at 37°C, to identify subpopulations at higher drug concentrations. Echinocandin MICs of subpopulations and main populations were assessed by broth microdilution.

Anidulafungin heteroresistance was found in all 10 *C. auris* isolates, and in 5/10 *N. glabratus* and 2/10 *C. albicans* isolates. *N. glabratus* anidulafungin heteroresistant subpopulations displayed increased anidulafungin and caspofungin MICs compared to the main susceptible population of same isolates. Micafungin MICs were unchanged between populations.

This study extends existing knowledge of echinocandin heteroresistance in *Candida* species, and highlights different responses to echinocandin drugs by heteroresistant subpopulations in *N. glabratus.* Future work will further explore these differences between echinocandin drugs in heteroresistant *C. auris* and underlying genetic resistance mechanisms in these subpopulations.

BLOCK B

Characterising a conserved E3 Ubiquitin ligase required for *Candida albican*s fitness in diverse stress conditions.

Zirong Zeng[1], Jakub Chrustowicz[2], Arno Alpi[3], Vasso Makrantoni[1,4]

[1]Centre for Inflammation Research, Institute for Regeneration and Repair, University of Edinburgh, United Kingdom. [2]Department of Molecular Machines and Signalling, Max Planck Institute of Biochemistry, Martinsried, Germany. [3]Institute for Cell Biology, University of Edinburgh, United Kingdom. [4]Zhejiang University-University of Edinburgh Institute, Zhejiang University School of Medicine, Haining, China

Abstract

Candida albicans is a leading cause of fungal infections in humans, with mortality rates as high as 40% despite treatment. In order to deal with the dynamic and contrasting niches within the host, *Candida* must rapidly adapt and efficiently assimilate all needed nutrients, including carbohydrates, amino acids, and metal ions present in these microenvironments. To achieve this, fungi regulate specific nutrient uptake mechanisms and rewire their metabolism, displaying an impressive degree of metabolic flexibility. This metabolic flexibility, which enhances the fitness of the fungus, is as essential for pathogenicity as fungal virulence factors, thereby representing an attractive target for potential therapeutic intervention. Cellular responses to metabolic stress stimuli are mediated through gene regulatory networks and posttranslational modifications. Increasing evidence suggests levels of regulation by the Ubiquitin Proteasomal System (UPS). Central to the UPS are E3 ubiquitin ligases targeting proteins with ubiquitin for their proteasomal degradation, thereby controlling protein amount and turnover. Here, we have combined genetics, biochemistry and omics-based approaches to characterised a conserved multiprotein E3 ubiquitin ligase complex required for metabolic stress response in *Candida albicans.*

BLOCK B

The opaque truth: which Candida albicans strains really mate?

Shani Lifshitz, Nora Kawar, Judith Berman

Tel Aviv University, Israel

Abstract

Candida albicans is usually an a/α diploid that does not mate. For mating to occur, strains must become MTL-homozygous or hemizygous (a, α, a/a or α/α), switch to the opaque state, and encounter a mating partner of the opposite mating type. The resulting mating products are tetraploid. Heterozygosity at the Mating Type Locus (MTL), specifically in genes encoding the a1/α2 heterodimeric transcription regulator, represses mating by locking cells in the white state. These cells are unresponsive to pheromones, lack mating gene expression, and do not form mating projections. Here, we are investigating mating competence in a large collection of *C. albicans* isolates to address two questions: (1) which strains are naturally MTL-homozygous?; and (2) which strains can mate efficiently under laboratory mating-inducing conditions? We combine predictions of MTL protein sequences with high-throughput assays of mating efficiency. To test mating activity, we are optimizing the induction of the opaque state for the >1700 strains in our Synergy collection using several induction protocols that work for lab strains. The workflow is designed to enable rapid, high-throughput evaluation of mating potential in all strains, which should identify broader patterns of C. albicans mating behavior. The mating status of all strains will be compared with the sequence analysis of all 5 genes in the MTL locus and with all other phenotypes measured for the Synergy collection. This will allow us to study possible connections between mating, genome plasticity and growth phenotypes on diverse media, drugs and other stresses.

BLOCK B

Understanding biophysical and biological aspects of disk diffusion assays

Nora Kawar, Judith Berman

The Shmunis School of Biomedicine and Cancer Research, Tel Aviv University, Israel

Abstract

Disk diffusion assays (DDAs) are widely used to determine antimicrobial susceptibility; however, the biophysical factors influencing drug diffusion and their interaction with cells in the assay are not completely understood. Traditionally, in DDAs with *Candida* species, the size of the zone of inhibition (ZOI) after 24h of growth is inversely proportional to the minimal inhibitory concentration (MIC). In addition, assessing the growth inside the ZOI after 48h can provide a measure of antifungal drug tolerance, defined as a subpopulation of cells that grow slowly at drug concentrations above the MIC via a mechanism distinct from that of drug resistance. DDAs also help identify rare heteroresistant isolates—transiently resistant or tolerant cells that usually arise via acquired aneuploidy. Here, we investigated whether minor alterations in DDA protocols affect assay reproducibility; specifically, we tested the effect of drug format, media composition, and cell growth on DDA outcomes. First, drug format (solution vs solid) had no significant effect on assay results. Second, agar concentration, medium volume, and cell density influenced drug diffusion. And third, cell density may limit the surface availability of the drug. Importantly, drug tolerance and heteroresistance were much more sensitive than susceptibility/resistance to the combinatorial effects of drug, medium, and cells. These studies help improve DDA reproducibility and the interpretation of mechanisms that permit the detection of antifungal drug tolerance as well as heteroresistance.

BLOCK B

Functional analysis of uncharacterized essential *Candida albicans* protein kinases

Bernardo Ramírez-Zavala, Johannes Lackner, Ines Krüger, Sonja Schwanfelder, Joachim Morschhäuser

Institute of Molecular Infection Biology, University of Würzburg, Germany

Abstract

Protein kinases are fundamental components of signal transduction pathways that regulate nearly all cellular processes. Using inducible gene deletion, we tested which protein kinases are essential for viability in *Candida albicans*. After forced deletion of the uncharacterized orf19.5376, the null mutants grew as highly elongated, often multinucleate cells that failed to separate and collapsed after several hours. The closest homolog of orf19.5376 in the model yeast *Saccharomyces cerevisiae* is *ELM1*, which encodes a non-essential protein kinase involved in the regulation of cell morphogenesis, septin organisation and cytokinesis. The terminal phenotype of orf19.5376Δ mutants indicated that the encoded kinase has related functions that are indispensable for viability in *C. albicans*. orf19.3456 does not have an ortholog in *S. cerevisiae*, but the encoded kinase is similar to Sid1 of the fission yeast *Schizosaccharomyces pombe*. Sid1 is a component of the septation initiation network (SIN), a pathway related to the *S. cerevisiae* mitotic exit network (MEN). After forced deletion of orf19.3456, the cells grew as aseptate, multinucleate hyphae that eventually lysed. The orf19.3456Δ mutant phenotype indicated that the encoded kinase is a functional Sid1 ortholog and that *C. albicans*, despite being a budding yeast like *S. cerevisiae*, possesses a signaling pathway that is more related to the SIN of *S. pombe* than to the MEN of *S. cerevisiae*. These results illustrate that inducible gene deletion is a powerful method to determine gene essentiality in *C. albicans* and, by observing the terminal phenotype of the null mutants, get insights into their function.

BLOCK B

Identifying genes linked to long-term viability in Candida albicans

Natali Matry, Marina Druseikis, Sofya Rabinovich, Judith Berman

Shmunis School of Biomedicine and Cancer Research, Tel Aviv University, Israel

Abstract

Quiescence is a temporary and reversible exit from the cell cycle and is thought to confer resilience to stress conditions, including exposure to drugs that kill most cells in a susceptible strain. Entry into the quiescent state often occurs via nutrient limitation and confers long-term viability during starvation. We posit that quiescent subpopulations may lead to antifungal treatment failures. To better understand mechanisms that promote survival under stress conditions, we screened a library of 758 Over Expression (OE) strains for those that may contribute to quiescence. We identified genes that, when overexpressed, yielded high or low viability phenotypes relative to the WT, as measured using Propidium Iodide (PI) staining. Several putative mutants with altered viability were confirmed using a lower-throughput Microcolony assay (MC) approach. To identify additional genetic factors that contribute to the resilience of *C. albicans,* we are now using an unbiased approach by screening a large transposon mutagenesis library and identifying genes that are enriched or depleted in long-term nutrient-limited conditions.

BLOCK C

The effect of lung colonisation with *Candida albicans* on *Staphylococcus aureus* infection

Tobias Köhler[1], Natalie E. Nieuwenhuizen[1], Wibke Böhnke[2], Maria Batliner[1], Annika Schöninger[1], Tobias Hertlein[3], Ilse D. Jacobsen[2], Knut Ohlsen[3], Oliver Kurzai[1,2]

[1]Institute for Hygiene and Microbiology, Germany. [2]Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Germany. [3]Institute for Molecular Infection Biology, Germany

Abstract

Background: The fungus *C. albicans* and the bacterium *S. aureus* are both commensal organisms that can cause severe systemic infections in immunocompromised patients, and are often co-isolated. However, whereas *S. aureus* can cause pneumonia in mechanically ventilated patients and disseminate from the lung, *C. albicans* almost never causes invasive lung infections. We aim to investigate the different behaviour of *C. albicans* and *S. aureus* in the lung, and the effect of *C. albicans* on *S. aureus* infection.

Methods: We established a novel lung colonisation/infection model using Balb/c mice. Mice were administered *C. albicans* intranasally at day 0 and infected intranasally with *S. aureus* on day 1. At day 2, lung, liver and kidney bacterial/fungal burdens as well as lung immune responses were analysed.

Results: After intranasal administration, *C. albicans* and *S. aureus* colony numbers declined though the *C. albicans* Δ*ece1* mutant persisted until day 5. Only *S. aureus* disseminated into the liver and kidney. This mimics the clinical situation where *C. albicans* does not cause invasive infection via the lungs, but *S. aureus* does. Short-term *C. albicans* and *S. aureus* colonisation primarily increased numbers of neutrophils and $CD11b^+$ dendritic cells in the lung. Cytokine analysis showed that IL-1β, IL-17 and Il-22 were sign. increased in *C.* albicans colonisation with subsequent *S. aureus* infection.

Conclusion: We successfully established a mouse model of *C. albicans* and *S. aureus* colonisation/infection in the lungs. IL-22 could be one decision point involved in the outcome of *S. aures* infections.

BLOCK C

Rescuing *Candida albicans* adhesion and stress resistance defects in yeast-locked strains

Chad Britton, Miguel Garcia, Derek Thomas, Ian Cleary

Grand Valley State University, USA

Abstract

When *Candida albicans* changes between yeast and hyphal forms the organization and composition of the cell wall changes. A number of hypha-specific genes that are expressed as part of that transition encode adhesins, including the extensive *ALS* family. We have previously shown that expressing *ALS3* in strains that are locked in the yeast form improves their ability to adhere to each other and to surfaces and to form biofilms. Here we show that expressing two other hyphal proteins, *DDR48* and *HWP1*, in strains with filamentation defects have more limited effects on adhesion in liquid culture than expressing *ALS3*. In addition, we observed that expressing *DDR48*, but not *ALS3* or *HWP1* can increase resistance to caffeine, which in part acts on the Tor pathway to regulate filamentation and hypha-specific gene expression. Taken together these observations reinforce the idea that in spite of structural similarities, hyphal surface adhesins have specialized functions in both adhesion and stress resistance.

BLOCK C

The nonpathogenic yeast *Debaryomyces hansenii* can contribute to inflammation with the support of *Candida albicans*

Ronny Martin[1], Nadja Thielemann[1], Oliver Kurzai[1,2,3]

[1]Institute for Hygiene and Microbiology, University of Würzburg, Germany. [2]National Reference Center for Invasive Fungal Infections, Leibniz Institute for Natural Product Research and Infection Biology- Hans Knoell Institute, Jena, Germany. [3]Research Group Fungal Septomics, Leibniz Institute for Natural Product Research and Infection Biology- Hans Knoell Institute, Jena, Germany

Abstract

Candida CTG species are frequent colonizers of the human gastrointestinal tract, and their intestinal enrichment can contribute to inflammatory bowel diseases but also to liver inflammation and fibrosis. Gut mycobiome data from affected patients indicate an increased abundance of the common food colonizer *Debaryomyces hansenii.* This member of the *Candida* CTG group is widely regarded as a non-pathogenic possibly only transient yeast with a growth optimum at 25°C. However, it was recently shown that *D. hansenii* contributed to impaired wound healing in Crohn's disease patients, indicating that this yeast indeed might survive in the gut and has pathogenic attributes. To further analyze this, we performed a comprehensive growth analysis of 14 environmental and clinical strains and found that six of them proliferated at 37°C. In addition, we analyzed the whole genomes of all tested strains and observed that five of these six isolates formed a distinct genetic cluster. Interestingly, they were also potent inducers of IL-17A release from freshly isolated T cells in an *ex-vivo* stimulation assay. Additionally, *D. hansenii* alone barely adhered to intestinal epithelial C2BBe1 cells, but the presence of *C. albicans* dramatically increased *D. hansenii* adherence to host cells. Further evidence for this *C. albicans*-mediated pathogenic potential of *D. hansenii* was indicated by significantly elevated IL-17A levels after a combined *ex vivo* T cell stimulation in comparison to single antigen stimulation with the same MOI. Our findings might explain how intestinal enrichment of both species could contribute to inflammatory properties of gut and liver disease.

BLOCK C

Components of the vacuolar iron export system influences fluconazole susceptibility and lipidome of Candida albicans biofilms

Carolina Pohl, Karabo Setsiba, Kelebogile Letebele, Olihile Sebolai, Jacobus Albertyn

University of the Free State, South Africa

Abstract

The past decades have witnessed a rapid increase in the development of resistance to widely used antifungal drugs. Interestingly, interference with iron homeostasis of *Candida albicans* has been reported to lead to increases fluconazole susceptibility. In yeasts, excess iron is stored in the vacuole, from where it can be released into the cytoplasm during time of scarcity. Genes involved in the transport of iron from the vacuole include *FET99* and *FTH2. FET99* encodes a multicopper oxidase responsible for the uptake and release of iron stored in the vacuole, while *FTH2* encodes a vacuolar iron permease. Importantly, the overall status of the membrane lipids serves as important determinant of drug susceptibility and the relationship between lipid metabolism and iron homeostasis may influence the pathogen's susceptibility to certain drugs. To explore this interaction, fluconazole sensitivity of Δ/Δ*fet99* and Δ/Δ*fth2* planktonic cells and developing biofilms was evaluated. These deletions caused a decrease in susceptibility. The lipid droplet, ergosterol content, lipidome and mitochondrial membrane potential of both mutants were also evaluated. It was found that the lipid droplet content was decreased in the mutant biofilms compared to their parental strain. In addition, the mitochondrial membrane, cell membrane and neutral lipidomes of the mutants were significantly influenced, possibly contributing to the decrease susceptibility by limiting fluconazole uptake. This work provides additional information regarding the interplay between lipid metabolism, iron homeostasis and fluconazole resistance in *C. albicans*, which may be exploited as novel drug targets.

BLOCK C

γ-clade TLO genes are required for RNAi-dependent repression of TLO gene expression in Candida albicans

Abed Alkarem Abu Al Haija, Derek Sullivan, Gary Moran

Dublin Dental University Hospital, Ireland

Abstract

Background: *Candida albicans* is a major fungal pathogen linked to opportunistic infections. Its *TLO* gene family, comprising 14 paralogs across α, β, and γ clades, encodes Med2, a component of the Mediator complex involved in transcriptional regulation, virulence, and antifungal tolerance. Evidence suggests RNA interference (RNAi) modulates *TLO* expression, though clade-specific roles remain unclear.

Objective: To explore regulatory and functional roles of individual *TLO* clades in the presence or absence of active RNAi using targeted CRISPR-Cas9 knockouts.

Methods: RNAi function in *C. albicans*SC5314 was restored by repairing a missense mutation in *AGO1*. Clade-specific *TLO* gene deletions were generated using CRISPR-Cas9 and validated by PCR. Mutants were assessed via growth assays, qRT-PCR, RNA-seq, biofilm formation, antifungal susceptibility, and metabolic profiling.

Results: Restoration of RNAi via *AGO1* repair had no significant effect on planktonic growth but led to reduced total *TLO* expression, indicating RNAi-dependent repression. Deletion of *TLO*γ genes resulted in a marked increase in total *TLO* transcript levels. Further analysis revealed that depletion of the *TLO*γ family to just 3 remaining alleles were sufficient to repress *TLO* expression in the presence of functional RNAi. In contrast, deletion of *TLO2* (the sole member of the β-clade) had no observable impact on growth or *TLO* gene expression under the conditions tested.

Significance: These findings highlight a specific role for the γ-clade in RNAi-dependent regulation of *TLO* expression. Ongoing work focuses on α-clade deletions to further clarify clade-specific contributions to *C. albicans* biology and pathogenesis.

BLOCK C

The impact of host metabolic environments on fungal physiology and antifungal drug susceptibility

Fiona Wahl[1], Andrea Lehmann[2], Jana Golcman[1], Laura Schander[1], Michael Mülleder[2], Markus Ralser[2], Joahnnes Hartl[1]

[1]Berlin Institute of Health at Charite, Germany. [2]Charité Berlin, Germany

Abstract

Fungal infections are a significant medical problem, with high mortality rates when they become invasive and affect the bloodstream. These infections face a limited repertoire of antimicrobials to which resistance is increasingly common. Furthermore, available diagnostics and in vitro susceptibility testing often fail to predict in vivo therapeutic outcome. One potential explanation is that current methodology does not adequately capture environmental factors and fungal physiology in infection sites.

While metabolism is one of the first contact points with the host and is crucial for growth and survival, metabolic host-pathogen interactions and their effects on antifungal treatment remain poorly understood. Here, we use available information on the molecular composition of human infection sites, growth assays, and metabolomics to identify small molecules that serve as nutrient sources in WHO priority pathogens. We find that many of these nutrients can be accessed by pathogenic yeast, and that adding metabolites abundant in the human host to common growth media drastically affects antifungal susceptibility. Mechanisms include direct metabolite-antifungal interactions; e.g. metabolites that share structural similarity with flucytosine reduce its uptake into cells and thus its efficacy. Furthermore, we find that fungal physiology changes in response to altered metabolic environments, which correlates with altered susceptibility, tolerance, and heteroresistance to the clinical antifungal fluconazole.

While the mechanistic basis remains a topic of ongoing investigation, our research highlights that environmental and metabolic factors are crucial to understanding antifungal drug action. Their understanding will be essential for improving infection prevention, diagnosis, and the development of future treatment strategies.

BLOCK C

Proteomic diversity of a clinical isolate collection of *Pseudomonas aeruginosa*

Nina Stremmel[1], Lisa Schlünz-Kahl[1], Ludwig Roman Sinn[1], Rachel Wheatley[2], Craig MacLean[3], Markus Ralser[1]

[1]Charité - Universitätsmedizin Berlin, Germany. [2]Queen's University Belfast, Germany. [3]University of Oxford, Germany

Abstract

Pseudomonas aeruginosa, a Gram-negative bacterium, thrives in various natural environments due to its versatile genome. As an opportunistic pathogen, it significantly contributes to hospital-acquired infections, particularly affecting immunocompromised and critically ill patients. Recent discoveries underscore the substantial genetic diversity within infecting *P. aeruginosa* strains, indicating that diversity within individual patients leads to significant resistance increases when confronted with antibiotic therapy. However, it is understood that virulence and antibiotic resistance is shaped not just by the genome but also by the metabolic states of bacteria. This study systematically phenotypes 27 clinical isolates and 2 reference strains. Assessment of fitness in different nutrient environments targeting central carbon metabolism demonstrates that isolates with few or no resistances do not exhibit fitness advantages in the tested conditions. Proteomic profiling of all isolates under standard conditions shows that, despite huge genetic diversity, their set of expressed proteins remains largely consistent. In contrast, several pathways show great variety in their expression across strains, among them is in the production of secondary metabolites that also serve as key virulence factors. We expect that investigating clinical strains in various conditions will lead to a better understanding of the role of metabolic programming in bacterial infections and ultimately help to develop treatment alternatives to antibiotics.

BLOCK C

Candida albicans adaptation to the murine gut implies permanent and transient chromosomal reorganizations that involves Flo8 lack of function

Prieto, Daniel[a]; Hidalgo-Vico, Susana[a]; Álvaro-Moya, Marina[a] ;Cortés, Isabel[a]; Blesa, Alba[a]; Maufrais, Corinne[b c]; Chistophe d´Enfert[b]; Alonso-Monge, Rebeca[a]; Pla, Jesús[a] and Román, Elvira[a]

a Departamento de Microbiología y Parasitología, Facultad de Farmacia, Universidad Complutense de Madrid, 28040 Madrid
b Institut Pasteur, Université Paris Cité, INRAE USC2019, Unité Biologie et Pathogénicité Fongiques, Département de Mycologie, 75015 Paris, France
c Institut Pasteur, Université Paris Cité, Hub de Bioinformatique et Biostatistique, Centre de Ressources et Recherche en Informatique (C2RI), 75015 Paris, France

Abstract

Background: *Candida albicans* adaptation to the mammalian gut implies specific mechanisms that are niche-specific.

Methods: We have obtained *Candida albicans* strains adapted to the gastrointestinal tract (GIT) using a commensalism model in mice. Genomic and phenotypic analyses, as well as analysis of the humoral response induced by these µEv strains has been performed.

Results: µEv strains have increased fitness to the GIT compared to the original wt strain in competition commensalism assays, show increased *WOR1* mRNA levels, do not filament *in vitro* and adhere better to the intestinal mucosa. However, *WOR1* deletion in µEv strains resulted in similar fitness compared to their isogenic non-deleted strains. Genome sequencing of µEv strains revealed, among other events, mutations in the *FLO8* transcription factor which was responsible for it deficient filamentation. *FLO8* reintegration also restored their bile salt susceptibility. µEv-pFLO8 strains showed 10-fold fungal loads compared to the parental strain in competition colonization assays although they were not able to outcompete CAF2 in the gut. µEv93 and *flo8* mutant strains induced an enhanced humoral response compared to a wt strain, that recognize both yeast and filament morphologies.

Conclusion: *C. albicans* adaptation to mammalian GIT involves permanent and transient chromosomal reorganizations, being the loss of *FLO8* partially responsible.

BLOCK C

Selection and validation of an optimal *Saccharomyces cerevisiae* strain as a probiotic for the treatment of vulvovaginal candidiasis

Mart Sillen[1], Silke Baldewijns[1], Ilse Palmans[1], Kar On cheng[2], Axel Dietschmann[2], Paul Vandecruys[1], Sarah Lebeer[3], Liesbeth Demuyser[1], Mark Gresnigt[2], patrick Van Dijck[1]

[1]Laboratory of Molecular Cell Biology, KU Leuven, Belgium. [2]Adaptive pathogenicity strategies, HKI Jena, Germany. [3]Environmental ecology and applied microbiology, U Antwerp, Belgium

Abstract

Vulvovaginal candidiasis (VVC) affects millions globally and is characterized by multifactorial immunopathology, with *Candida albicans* virulence driving disease progression through epithelial tissue damage and hyperactivated neutrophils contributing to disease severity. Probiotics like *Saccharomyces cerevisiae* show potential in mitigating such immunopathologies. Here, we explored the multifaceted nature of *S. cerevisiae* as a probiotic to attenuate *C. albicans* virulence and modulate host immune responses during VVC. Co-culture experiments demonstrated that *S. cerevisiae* targets multiple aspects of *C. albicans* virulence, including fungal proliferation, adhesion, and hyphal morphogenesis, collectively impairing biofilm formation and disrupting pathogenic potential. These effects were linked to transcriptional reprogramming in *C. albicans*, marked by metabolic stress and downregulation of virulence- and biofilm-related genes. Additionally, *S. cerevisiae* reduced epithelial inflammatory responses and neutrophil hyperactivation, while preserving neutrophil antimicrobial functionality. Mice experiments show the in vivo efficacy and show the clear dampening effect on the host immune system. Collectively, these findings establish *S. cerevisiae* as a promising probiotic therapy to mitigate *C. albicans* infection and alleviate inflammatory complications in VVC.

BLOCK C

Dissecting the ultrastructure of spindle pole bodies in the human fungal pathogen *Candida albicans*

Banishree Sahoo[1], Romita Basak[2], Kaustuv Sanyal[1,2]

[1]Molecular Mycology Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bangalore, 560064, India.
[2]Department of Biological Sciences, Bose Institute, Unified Academic Campus, EN-80, Sector V, Bidhannagar, Kolkata, 700091, India

Abstract

The spindle pole body (SPB), the functional equivalent of centrosomes, which acts as the microtubule organizing centre, is poorly studied in *Candida albicans* and its related species. Although SPBs are functionally conserved, several studies suggest that SPB components are rapidly evolving in fungi. By integrating functional genetics and proteomics approaches, we sought to dissect the structure and function of SPBs in *C. albicans.* Csa6, a recently identified CUG-Ser1 clade-specific SPB protein, is required for exiting mitosis and induces metaphase arrest when overexpressed. To understand the intricate details of the SPB structure and function, we employed a two-pronged approach. First, we analysed Csa6, which shows a separation of function cell cycle stage-specific domain function, suggestive of its multiple interacting partners at the SPB. Second, immunoprecipitation followed by mass spectrometry (IP-MS) analysis with Csa6 as the bait in an asynchronous culture of *C. albicans* identified the evolutionarily conserved SPB components, Spc110, Cmd1, and Cdc31, interacting with Csa6. Two other Csa6-associating proteins, Cap1 and Cap2, with unknown function, were also identified. Cap1 and Cap2 are phylogenetically restricted only in a few *Candida* species. *In vivo* analysis confirmed that both proteins are exclusively localized to SPBs. Preliminary functional analysis suggests Cap1 is not essential for viability. Further studies are underway to characterize their function at the SPBs. In summary, the presence of Csa6, Cap1, and Cap2 provides evidence for the existence of an evolutionarily diverged SPB structure. SPB components uniquely present in fungal pathogens are attractive targets for developing safer and specific antifungal drugs.

BLOCK C

The genomic landscape of acquired drug resistance in *Candida parapsilosis* complex

Vladislav Biriukov[1,2], Álvaro Redondo-Río[2,1], Juan Carlos Nunez-Rodriguez[1,2], Mrinalini Parmar[1,2], Islam Ahaik[1,2], Toni Gabaldón[1,2,3,4]

[1]Institute for Research in Biomedicine (IRB Barcelona), Spain. [2]Barcelona Supercomputing Centre (BSC-CNS), Spain. [3]Catalan Institution for Research and Advanced Studies (ICREA), Spain. [4]Centro de Investigación Biomédica En Red de Enfermedades Infecciosas (CIBERINFEC), Spain

Abstract

Invasive infections caused by the *Candida parapsilosis* species complex are a serious threat due to rising azole resistance and low intrinsic susceptibility to echinocandins, frequently resulting in deadly hospital outbreaks. However, the genomic mechanisms driving antifungal adaptation in this complex remain largely unexplored.

We performed large-scale in vitro evolution in increasing concentrations of fluconazole, anidulafungin, and their sequential or combined treatments on 12 diverse strains of *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis*. Whole-genome sequencing of 112 evolved strains revealed novel mutations, CNVs, and aneuploidies underlying antifungal resistance.

Nearly 90% of fluconazole-evolved strains carried *MRR1* mutations, including 42 novel variants, mainly clustered within two regions, suggesting new gain-of-function hotspots. We also identified several compensatory loss-of-function mutations associated with decreased resistance under fluconazole-to-anidulafungin treatment. Anidulafungin resistance was driven mainly by *FKS1* mutation (~97% of resistant strains), mostly within known hotspots HS1 and HS2. A mutation in a rarely reported HS3 conferred high-level resistance. Nine mutations occurring outside these regions also conferred reduced susceptibility. Besides key drivers, we identified recurrent mutations in transcription factors and stress-response pathway genes, contributing to fluconazole and anidulafungin resistance. Aneuploidies and CNVs frequently emerged under antifungal treatment, particularly with fluconazole, often spanning known resistance genes, and were frequently reverted under sequential treatment, confirming their high genomic plasticity.

This study reports the most comprehensive genome-wide analysis of antifungal adaptation in the *C. parapsilosis* complex. We showed that resistance emerges from an interplay between SNPs, CNVs, and aneuploidies, confirming the advantage of WGS strategies over gene-focused methods.

BLOCK C

Mutational profiling of the Rta-family of proteins in *Candida albicans* uncovers critical regions for activity

Prerna Pathak, Darakshan Alim, Sneh Panwar

Yeast Molecular Genetics Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

Abstract

Background: Rta2 and Rta3, the *C. albicans* Rta1-family of proteins regulate membrane lipid composition, and confer ER stress resistance. The Rtas consists are membrane proteins with a conserved signature sequence consisting of 28 amino acids situated between the sixth- and seventh-transmembrane domains; hallmark of these proteins.

Methods: For splicing assays, extracted RNA from cells treated with tunicamycin was subjected to cDNA synthesis, followed by RT-PCR with *HAC1* gene-specific primers. For flippase activity, cells labeled with 5µM NBD-PC were washed, kept at 30°C for 30 minutes, followed by final washing in cold SC-azide.

Results: Herein, we attempted to identify regions crucial for the activity of the Rta proteins. We used the ability of Rta3 to regulate the flipping of NBD-labeled phospholipids across the plasma membrane, and the kinetics of Rta2-mediated splicing of *HAC1* mRNA as parameters to analyze the impact of the site-directed mutations. Our study focused on three regions of this protein, namely, the signature motif, loop 5, and C-terminus. We demonstrate the essentiality of the signature sequence, and loop 5 for the stability, and activity of the Rtas. Notably, the signature sequence and the C-terminal region of Rta2, was shown to be required for the Rta2-mediated attenuation of the ER stress-induced unfolded protein response (UPR) in the fungus.

Conclusion: Owing to their exclusive presence in the fungal kingdom, targeting Rta proteins may have therapeutic potential. We surmise that targeting the conserved signature sequence, and loop 5 will impede the function of the Rta proteins.

BLOCK C

Functional characterization of a novel gene important for pre-mRNA splicing in *Candida albicans*

Livia Kupczok[1], Ci Fu[2], Linda S. Archambault[2], Sofia Maroccia[2], Nicole Robbins[2], Leah E. Cowen[2], Lydia Herzel[1]

[1]Freie Universität Berlin, Germany. [2]University of Toronto, Canada

Abstract

The ability of the human fungal pathogen *Candida albicans* to adapt to diverse environmental conditions is critical for its ability to thrive in a human host. This requires numerous stress response pathways that are tightly regulated by changes in gene expression. In addition to changes in the transcription of genes, post-transcriptional changes at the level of RNA, e.g. by pre-mRNA splicing, form another layer of possible gene regulation, but are not well understood in *C. albicans*. Pre-mRNA splicing, which is the removal of introns from intron-containing pre-mRNAs, is catalysed by the spliceosome. Even though less than 6% of genes in the *C. albicans* genome contain an intron, spliceosomal genes are often essential and their perturbation can lead to impaired filamentation, a key virulence trait (Lash *et al.* mBio 2024).

Here, we identified a previously uncharacterized gene (*C1_11680C* or *YSF3*) as a component of the *C. albicans* spliceosome, which is involved in the recognition of intronic sequence elements to prepare for catalysis. Genetic perturbation revealed severe growth phenotypes across a wide temperature range. Global pre-mRNA splicing was strongly impaired upon *YSF3* depletion and especially perturbed for introns with non-consensus sequence elements, as measured by RNA-seq. Furthermore, the amount of unspliced pre-mRNA increased by more than 4-fold. Translation of such 'faulty' messages could produce incomplete, possibly toxic and misfolded proteins that could impact growth. Consistently, proteasomal genes were strongly upregulated upon this perturbation. Overall, these findings provide significant advance in our understanding of post-transcriptional regulation in *C. albicans* governing fungal viability.

BLOCK C

Identifying and characterizing *Candida albicans* genes that modulate susceptibility to antifungals

Veronika Pavlova, Nicole Robbins, Leah Cowen

University of Toronto, Canada

Abstract

Candida albicans is the leading cause of invasive candidiasis, resulting in life-threatening infections in immunocompromised individuals. Antifungal therapies are limited to three main drug classes: polyenes, azoles, and echinocandins. These target either the fungal membrane component ergosterol, ergosterol biosynthesis, or biosynthesis of the cell wall component β-1,3-glucan, respectively. Resistance to antifungal treatments poses an increasing concern, and with the limited treatment options, there is an urgent need to develop new therapeutic strategies. Manogepix (MGX) is a novel antifungal currently undergoing phase 3 clinical trials, representing the gepix drug class that functions by inhibiting fungal glycosylphosphatidylinositol (GPI)-anchor biosynthesis. Nikkomycin Z (NikkZ) is a compound also being investigated for its antifungal properties, which inhibits fungal chitin synthases. To date, the genes important for susceptibility to MGX and NikkZ are largely enigmatic. This project aims to identify and characterize *C. albicans* genes that modulate susceptibility to diverse antifungals. To do so, we leveraged a large-scale collection of *C. albicans* Gene Replacement and Conditional Expression mutants with regulatable gene expression through a doxycycline-repressible promoter. Specifically, we identified genes that modulate susceptibility to the echinocandin caspofungin, the azole fluconazole, NikkZ, and MGX. Further investigation of two genes, *SNF6* and *PAT1*, for which transcriptional repression conferred hypersensitivity to MGX, implicated the SWI/SNF complex as a general regulator of antifungal stress and Pat1 as a specific regulator of MGX-induced stress through P-body formation. Overall, this work expands our understanding of genes that influence antifungal responses, which is crucial for developing effective treatments and overcoming resistance.

BLOCK C

The Galleria mellonella–Candida albicans Model: A Tool for Studying Host–Pathogen Interactions and Antifungal Drug Efficacy

Margy Alejandra Esparza Mora, Mariella Obermeier, Markus Ralser

Charité – Universitätsmedizin Berlin, Institut für Biochemie, Germany

Abstract

Candida albicans is the most common opportunistic human pathogen that causes significant mortality among immuno-compromised patients. This fungal pathogen also causes lethal infections in insects such as Galleria mellonella, following hemolymph injection. G.mellonella is widely used as an in vivo infection model to study fungal pathogenesis, host immune responses and the efficacy of anti-Candida agents. In this study, G. mellonella larvae were used to investigate the host response to C. albicans infection and evaluate antifungal antagonism. We found that an inoculum of 1x10^6 CFU/larva resulted in 100% mortality within 3 days, while 5x10^5 CFU/larva caused 100% mortality within 5 days. To assess antifungal efficacy, fluconazole (FLC) was selected. G. mellonella exhibited dose-dependent protection against C. albicans infection following treatment with FLC at concentrations of 0.5, 1, 4, and 16 mg/kg. Hemocyte density was meassured to assess the cellular response during fungal infection, revealing a significant reduction at both 12 and 24 hours post-infection, regardless of PBS or FLC treatment. In vitro, FLC demonstrates potent antagonistic interactions when combined with several drugs commonly administered to patients at high risk for fungal infections. To determine whether these interactions persist in vivo, we used the G. mellonella–C.albicans model to test combinations of FLC with estradiol, levothyroxine, and loperamide. These combinations resulted in increased larval mortality, indicating potential in vivo antagonism. A better understanding of the molecular mechanisms underlying such antagonistic interactions, and their clinical relevance, will be critical for optimizing antifungal therapies and mitigating adverse drug interactions.

BLOCK C

Expansion of the Gene Replacement and Conditional Expression (GRACE) library identifies regulators of *Candida albicans* pathobiology

Yunjin Lee[1], Ci Fu[1], Pauline Basso[2], Linda Archambault[1], Nicole Robbins[1], Suzanne Noble[1], Leah Cowen[1]

[1]University of Toronto, Canada. [2]University of California San Francisco, USA

Abstract

Fungal pathogens pose a devastating impact on global health, with *Candida albicans* representing a major cause of systemic disease in immunocompromised patients. Only three major classes of antifungal drugs are available in the clinic to treat these life-threatening infections. With antifungal resistance on the rise, expanding our knowledge of the genetic circuitry mediating fungal pathobiology is important to identify additional therapeutic targets. Functional genomic analysis provides a powerful strategy to interrogate gene function and reveal insights into the vulnerabilities of *C. albicans*. The Gene Replacement and Conditional Expression (GRACE) library is a large-scale *C. albicans* mutant library consisting of heterozygous deletion strains where the expression of the remaining allele is under the control of a doxycycline (DOX)-repressible promoter. Originally, the library provided ~37% coverage of the 6,198 genes in the *C. albicans* genome; previously we expanded the collection to ~48% coverage (termed GRACEv2). Here, we introduce an additional 1,240 GRACE strains to the collection, expanding the coverage of the *C. albicans* genome to ~71% (termed GRACEv3). Systematic screening of the expanded GRACE library under diverse *in vitro* conditions uncovered genes important for fitness in multiple environments and enabled the interrogation and characterization of previously unknown genes. In addition, pooled screening of the expanded library in mouse models of disseminated candidiasis and commensalism revealed genes important for survival in a mammalian host. Collectively, this work leverages the largest mutant library in *C. albicans* to unveil critical insights into the pathobiology of this important pathogen.

BLOCK C

In vivo evolution of *Candida auris* multi-drug resistance in a patient receiving antifungal treatment

Tristan Wang, Nicole Putnam, J. Kristie Johnson, Mary Ann Jabra-Rizk

University of Maryland, Baltimore, USA

Abstract

Candida auris is a newly emerged human fungal pathogen associated with an exponential rise in invasive candidiasis with high mortality rates. Most concerning is the unique ability to develop multidrug resistance to all main classes of antifungals. A striking morphological feature of some *C. auris* clinical isolates is their capacity to aggregate and form strong biofilms, an important risk factor for systemic infections. In this study, we present a case where *C. auris* isolates from a patient receiving antifungal treatment acquired resistance to caspofungin and amphotericin B over the course of receiving antifungal therapy. Five isolates were prospectively recovered from a transplant patient receiving antifungal therapy over a one-year period. While all isolates were initially only resistant to fluconazole, the terminal isolate became resistant to caspofungin and amphotericin B. Amplification of the *ITS* and *RHA1* genes identified isolates as belonging to clade 1.Sequencing of *ERG11* and *FKS1* genes identified mutations associated with fluconazole (Y132F) and echinocandin (F635Y) resistance in the multi-drug-resistant isolate but no known mutations were identified in *ERG6* gene, indicating alternative mechanisms of resistance to amphotericin B. Surprisingly, although recovered from the same patient, the isolates displayed large phenotypic variations in growth rates, ability to aggregate, and form biofilms suggesting possible changes to transcriptional regulation that may impact pathogenic potential. Combined, these findings underscore the alarming threat of therapy-induced development of resistance and highlight the plasticity and adaptability of *C. auris* with potential implications to the host.

BLOCK C

Identification and characterization of the adamantanyl diamine SQ109 as a repurposed inhibitor of squalene synthase in the fungal pathogen *Candida albicans.*

Sara Fallah[1], Xuefei Chen[2], Sunna Nabeela[3], Robert Zarnowski[4,5], Luke Whitesell[1], Priya Uppuluri[3], David Andes[4,5], Gerard Wright[2], Nicole Robbins[1], Leah Cowen[1]

[1]Department of Molecular Genetics, University of Toronto, Canada. [2]Department of Biochemistry and Biomedical Sciences, McMaster University, Canada. [3]Lundquist Institute for Biomedical Innovation at Harbor-University of California Los Angeles Medical Center, USA. [4]Department of Medicine, University of Wisconsin School of Medicine and Public Health, USA. [5]Department of Medical Microbiology and Immunology, University of Wisconsin, USA

Abstract

Candida albicans is a leading cause of deadly fungal infections in a growing population of immunocompromised patients. Alarmingly, the efficacy of azoles, the most common antifungal class used to treat these infections, is increasingly limited by the development of resistance. Screening of well-annotated chemical libraries offers a powerful approach to uncover molecules for repurposing as antifungals, expediting drug development and lowering regulatory barriers and cost. Thus, in a quest to identify novel antifungals, we screened the Medicines for Malaria Venture's library of 640 compounds in various stages of drug development. Through this approach we identified the antitubercular drug SQ109 as a molecule with broad-spectrum activity against *C. albicans*, *Nakaseomyces glabratus*, *Cryptococcus neoformans, Aspergillus fumigatus,* and *Trichophyton indotineae.* Mechanistic studies indicated that SQ109 inhibits the *C. albicans* squalene synthase Erg9, as compound treatment increased lipid droplet accumulation, indicative of altered lipid homeostasis, and decreased the sterol intermediates, squalene and lanosterol as well as ergosterol itself. While robust Erg9 inhibition killed *C. albicans*, sublethal concentrations of SQ109 impaired *C. albicans* virulence traits including filamentation and biofilm formation. In co-culture assays with mouse monocyte-macrophage lineage J774A.1 cells, treatment with sub-growth inhibitory concentrations of SQ109 rescued macrophage viability through the inhibition of intraphagosomal *C. albicans* filamentation. Work is in progress to explore the therapeutic potential of SQ109 in a mouse model of *Trichophyton mentagrophytes* dermatomycosis and in a rat catheter model of *C. albicans* biofilm formation. Overall, this work characterizes SQ109 as a promising antifungal candidate with both broad-spectrum and anti-virulence activities.

BLOCK C

Regulation of polymer formation of the Candida albicans virulence factor candidalysin

Teresa Rodríguez-García[1], Sadie L. Nickles[2], Gavin M. King[2], Francisco N. Barerra[1]

[1]University of Tennessee, USA. [2]University of Missouri, USA

Abstract

Candida albicans secretes candidalysin (CL), a peptide that causes cell damage and immune activation by permeation of epithelial membranes. A recent genome-wide loss-of-function CRISPR screen identified that disruption of genes in glycosaminoglycan (GAG) biosynthesis conferred resistance to damage induced by both CL and live *C. albicans*, but the specific mechanism behind this behavior is poorly understood. CL action involves peptide assembly into polymers in solution. We hypothesize that the free ends of linear CL polymers can join, forming loops that become pores upon binding to membranes. CL polymers constitute a therapeutic target for candidiasis, but the mechanism of CL self-assembly in solution is poorly understood. Biophysical experiments revealed that CL polymerization involves a convolution of four processes. Self-assembly begins with the formation of a basic subunit, thought to be a CL octamer. Polymerization proceeds via the addition of octamers, and as a polymer grows it can curve and become a loop. Alternatively, secondary polymerization can cause branching. Interplay between the rates for these processes determines the distribution of CL particle types, indicating that CL polymerization is under kinetic control. The presence of GAGs influences the polymerization of CL. Specifically, GAG analogs enhance the self-assembly of candidalysin in solution. We grafted GAGs to the surface of synthetic lipid vesicles, and observed that GAG also impact pore formation. The results suggest that regulatory mechanism might be needed for productive CL polymerization leading to pores. This work elucidates key physical attributes underlying CL self-assembly which may eventually evoke pharmaceutical development

BLOCK C

RGD motif-dependent internalization of *Candida albicans* Sap6 protease defines neutrophil response and immune evasion outcome

Marcin Zawrotniak, Dorota Satala, Grazyna Bras, Maria Rapala-Kozik

Jagiellonian University, Poland

Abstract

Background

The aspartyl protease Sap6 secreted by *Candida albicans* contains tandem RGD motifs that enable binding to neutrophil surface integrins and potentially modulate their function. Neutrophils are the first line of defense in fungal infections, but the mechanisms by which Sap6 affects their activity remain incompletely understood.

Methods

Flow cytometry and fluorescence microscopy were used to analyze Sap6–neutrophil interactions, including binding, internalization, and downstream functional effects. Neutrophils were phenotyped based on CD62L, CXCR2, and CXCR4 surface expression. Caspase 3/7 activation, NET release, reactive oxygen species (ROS) production, and NADPH oxidase subunit degradation were assessed. Synthetic RGD peptides were used to confirm the sequence-specific role of Sap6 binding.

Results

We found that Sap6 binds to Mac-1 integrin and undergoes active, RGD-dependent internalization. Intracellular accumulation of Sap6 led to the degradation of $p67^{phox}$ and $gp91^{phox}$ subunits and resulted in strong suppression of ROS production and NETosis. Blocking the RGD–integrin interaction reduced Sap6 uptake and preserved neutrophil effector functions. Notably, the cellular response to Sap6 varied between neutrophil subsets and correlated with surface marker profiles, suggesting that cell phenotype or maturity influences susceptibility.

Conclusion

Sap6 utilizes the RGD motif for selective binding and internalization into neutrophils. Its proteolytic activity disrupts oxidative responses by degrading key components of the NADPH oxidase complex. The described mechanism represents a novel immune evasion strategy of *C. albicans*, in which the fungus can selectively inactivate innate immune cells under specific physiological conditions.

BLOCK C

Genetic Basis of Amino Acid Metabolism in Yeast

Natalie Barthel[1,2], Christopher Jakobson[3], Johannes Hartl[1], Markus Ralser[1]

[1]University Hospital Charité, Germany. [2]Free University Berlin, Germany. [3]University of Stanford, USA

Abstract

Understanding how genetic variation shapes amino acid metabolism is crucial, as metabolic adaptations often underlie mechanisms of drug resistance and drug and stress tolerance in fungi. A recent study comprehensively assessed the yeast genome's metabolic functions by measuring amino acid levels in 5,000 knockout strains, revealing the extensive interplay between amino acid metabolism and cellular processes (Mülleder et al., 2016). However, the impact of natural genetic variation on metabolism remains unclear, a gap that we address with genetic mapping using natural budding yeast strains. Here, we used targeted high-throughput metabolomics on 851 segregants from intercrosses of two wild *Saccharomyces cerevisiae* strains, RM11 and YJM975 (She et al., 2018). We observed striking metabolic diversification between the parents and their progeny, comparable to natural variation across wild isolates. This study generated a genotype-to-metabolite atlas, identifying over 1,100 genetic loci shaping amino acid levels. Some loci corresponded to known metabolic enzymes, such as a *PHA2* variant affecting phenylalanine, while others implicated uncharacterized genes as novel regulators of metabolism. By integrating mutation-to-metabolite and mutation-to-protein maps, we uncovered mechanisms of global metabolic regulation, including the RAS GTPase activator IRA2/NF1. Besides, we revealed a new role for an unnamed prolyl-tRNA synthetase. Our findings reveal key genetic and regulatory factors underlying metabolic variation, enhancing our understanding of cellular adaptation in challenging environments—a process central to drug resistance and laying the groundwork for exploring similar mechanisms in opportunistic pathogens like *Candida*.

BLOCK C

Intrahost Diversity of Candida Species from Invasive and Commensal Samples Analyzed via Isolates and Metagenomics

Brooks Burt[1], Terrance Shea[1], Hannah Snell[1], Daniel Floyd[2], Adam Viens[2], Johnny Atallah[2], Anna Apostolopoulou[2], Michael Mansour[2], Christina Cuomo[1]

[1]Infectious Disease and Microbiome Program, Broad Institute, USA. [2]Division of Infectious Diseases, Department of Medicine, Massachusetts 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* during the course of these at-risk infections. We characterized the diversity of *Candida* present in patients with candidemia, comparing bloodstream isolates to that found at other body sites and reservoirs. We carried out whole genome sequencing (WGS) on cultured blood stream isolates, whole metagenomic sequencing (WMS) on stool samples, and internal transcribed spacer (ITS) sequencing on stool and skin swab samples to amplify fungi. *Candida albicans*, *Candida glabrata*, and *Candida parapsilosis* were most commonly detected, with additional cases of many other fungal species. We found that blood isolates (WGS) from the same patients are highly identical to each other, although some displayed ploidy variation and loss of heterozygosity, and to *Candida* from stool (WMS), however 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. A complete, phased genome was assembled of a *C. orthopsilosis* isolate that identifies the origin of the two parental genotypes. 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.

BLOCK C

Role and regulation of Candida albicans dispersal

Eli Cytrynbaum, Megan McClean

University of Wisconsin - Madison, USA

Abstract

The ability to switch between cellular states with distinct metabolic signatures, adhesins, and morphologies enables *Candida albicans* to invade a variety of host tissues while evading the immune system as well as common treatments. Cell state transition has also been linked to commensal host immune and metabolic regulation. Understanding these cell transitions is crucial, but dispersal–the process by which hyphal dominated biofilms generate planktonic yeast-form cells–remains poorly understood. Rather than occurring constitutively, we discovered that dispersal from biofilms occurs as a discrete event within biofilm development that coincides with the cessation of biofilm expansion. We found that both dispersal and the formation of new biofilms from dispersed cells is regulated by intercellular signaling in addition to environmental conditions. We observed that dispersed cells are morphologically heterogenous. Ongoing work is elucidating the signaling networks driving this heterogeneity, as well as its impacts on biofilm reseeding and immune response. By modeling the regulatory pathways controlling dispersal, we hope to uncover potential targets for treatment to encourage commensalism over pathogenesis.

BLOCK C

A transcription factor that governs the *Candida albicans* response to different host cells

Norma Solis[1,2], Katharina Goerlich[3], Aaron Mitchell[3], Scott Filler[1,2,4]

[1]Division of Infectious Diseases, Harbor-UCLA Medical Center, USA. [2]The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, USA. [3]University of Georgia, USA. [4]David Geffen School of Medicine at UCLA, USA

Abstract

C. albicans strain SC5314 damages both human umbilical vein endothelial cells (HUVECs) and oral epithelial cells (OECs) *in vitro*. It is rapidly lethal in the mouse model of hematogenously disseminated candidiasis (HDC). SC5314 also elicits a strong inflammatory response in the mouse model of oropharyngeal candidiasis (OPC), leading to rapid clearance from the oropharynx. *C. albicans* strain CEC3672 damages HUVECs, but not OECs *in vitro*. Although CEC3672 is virulent in the mouse model of HDC, it elicits a weak inflammatory response during OPC, leading to persistence in the oropharynx. To identify potential mechanisms for these differences, we performed RNA-seq analyses. We found that the transcriptional response of SC5314 to HUVECs and OECs was very similar, with only 37 differentially expressed genes between the two cell types. CEC3672 was much more responsive to the host cell type, with 217 differentially expressed genes. Notably, exposure of CEC3672 to OECs caused downregulation of virulence genes, including *ECE1, ALS3, SSA2, PRA1,* and *SAP9,* explaining its reduced capacity to damage OECs. This analysis identified the transcription factor LYS143 as a possible repressor of virulence gene expression. Overexpression of *LYS143* in SC5314 and CEC3672 reduced damage to HUVECs by 31% and 55%, respectively. Overexpression of *LYS143* in SC5314 also reduced OEC damage by 85%. RNA-seq analysis showed that overexpression of *LYS143* in SC5314 significantly reduced the expression of *ECE1, ALS3, SSA2, SAP6,* and *SAP9.* Thus, LYS143 is a negative regulator of host cell damage and may help induce commensalism in *C. albicans.*

BLOCK C

Mixed population of echinocandin-susceptible and -heteroresistant Candida auris strains from a patient with multi-focal infection

Shaoji Cheng, Cornelius Joseph Clancy, Minh Hong Thi Nguyen

University of Pittsburgh, USA

Abstract

Background: Antifungal heteroresistance (HR), in particular azole-HR, is described in Nakaseomyes glabrata, Candida parapsilosis and Cryptococcus neoformans; echinocandin-HR is poorly characterized. We have shown that blood cultures from patients with N. glabrata and C. albicans bloodstream infections are comprised of clonal, but genetically diverse strains, some of which may differ in antifungal responsiveness. C. auris strains are typically azole-resistant, echinocandin-susceptible. We hypothesized that C. auris infections in some patients are caused by a mixed population of echinocandin-susceptible and -HR strains.

Methods: We collected 20 C. auris strains from 4 sites in a patient (mediastinum; chest hematoma; bronchoalveolar lavage (BAL); wound; n=5 each). We used population analysis profiling to measure micafungin-HR. Strains underwent Illumina whole genome sequencing.

Results: Strains did not differ by colony morphology, growth rate in liquid YPD, plastic adhesion or biofilm formation. Strains were fluconazole-resistant, voriconazole-, amphotericin B-, micafungin- and caspofungin-susceptible by minimum inhibitory concentrations (MICs). A strain from BAL was micafungin-HR. Micafungin-resistance frequency at 16 µg/mL was 0.5%. Colonies grown on Micafungin 16ug/ml were susceptible (MIC=0.5 µg/mL). Strains belonged to clade I. SNP distances among strains were 0-3. Compared to other strains, the Micafungin-HR strain has 6 unique InDels and structural variants, 5 of which are intergenic (2 insertions, 3 deletions). 18bp deletion in CJI96_0000459 resulted in 88Q>H, 89-95 QHHGGG deletion.

Conclusions: Micafungin-HR strains may be present within a micafungin-susceptible C. auris population. Mechanisms of micafungin-HR are currently under investigation.

BLOCK C

The antifungal activity of Cinnamomum zeylanicum and Syzygium aromaticum essential oils against Candida Species isolated from patients admitted to Tertiary Care Hospitals in Lahore, Punjab, Pakistan.

Kashif Sadiq, Shahan Azeem, Aftab Ahmad Anjum, Muhammad Muddassir Ali

Institute of Microbiology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Pakistan

Abstract

The present study was designed to evaluate the presence of Candida species, their antifungal resistance, and the antifungal activity of *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils against local isolates of Candida. Briefly, a total of 25 (urine, vaginal swab, follies catheters tips, wound pus, and throat swab) samples were collected from four tertiary care hospitals in Lahore, Punjab, Pakistan. The samples were cultured on Sabouraud Dextrose Agar, followed by germ tube and rapid urease tests. The putative Candida-positive samples were subjected to PCR and gene sequencing targeting a 221 bp segment of 18S rRNA. All Candida-positive samples were further evaluated by *in vitro* antifungal resistance, followed by antifungal activity of *Cinnamomum zeylanicum* and *Syzygium aromaticum* essential oils. Ten samples were positive for Candida with a positivity rate of 40% (10/25). Gene sequencing confirmed *Candida albicans* in all the tested samples. The antifungal sensitivity testing demonstrated that Clotrimazol was the most sensitive drug against all isolates and Voriconazole was the least sensitive. The *in vitro* antifungal evaluations of essential oils suggested that *Cinnamomum zeylanicum* oil had a broader zone of inhibition (20.8 mm–40.7 mm) compared to *Syzygium aromaticum* oil (10.8 mm–20.0 mm). However, the minimum inhibitory concentration of the two essential oils was not significantly different ($p>0.05$). The present study suggests the emergence of antifungal resistance in Candida species, posing a public health risk in healthcare settings. Bioassays could further evaluate the therapeutic potential of Cinnamon and Clove essential oils as alternative antifungal agents against *Candida albicans*.

BLOCK C

Polymicrobial biofilms enhance hypoxia-driven colonization and suppress epithelial apoptosis in lung adenocarcinoma cells

Ewelina Wronowska, Ibeth Guevara-Lora, Grazyna Bras, Marcin Zawrotniak, Dorota Satala, Justyna Karkowska-Kuleta, Maria Rapala-Kozik

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

Abstract

Mixed microbial infections are increasingly recognized as contributors to tumor microenvironment remodeling. In particular, interactions between *Candida albicans* and *Porphyromonas gingivalis* may influence immune evasion, epithelial barrier disruption, and cancer progression. However, their impact on lung cancer cells remains poorly defined.

To assess the role of fungal cell wall components in conditioning the microenvironment, we employed *C. albicans* deletion mutants deficient in mannan or β-glucan biosynthesis. A549 adenocarcinoma cells were exposed to dual-species biofilms in ALI and 3D models. Epithelial responses were evaluated by immunofluorescence (ZO-1, E-cadherin, claudin-1), ELISA (IL-8), and Western blot for oxidative stress and apoptosis markers (HO-1, Bax/Bcl-xL, caspase-3).

Wild-type *C. albicans* biofilms lowered local oxygen levels, enabling *P. gingivalis* growth even in aerobic settings. Mutants lacking mannans or β-glucans failed to support comparable bacterial proliferation. Hypoxia-driven synergy promoted colonization and penetration of the lung cancer epithelium. Dual-species infections significantly compromised barrier function in A549 cells (reduced ZO-1, delocalized claudin-1), suppressed IL-8 release (via gingipains), and altered stress responses—HO-1 was downregulated, and the Bax/Bcl-xL ratio shifted toward survival. The wild-type *P. gingivalis* strain W83 caused greater epithelial disruption than the gingipain-deficient mutant ΔKΔRAB.

Fungal biofilms rich in mannans and glucans create hypoxic conditions that support anaerobic bacterial adaptation. This cooperation enhances *P. gingivalis* colonization and promotes immune evasion and apoptosis resistance in lung cancer cells, highlighting a potential role for polymicrobial dysbiosis in tumor progression.

BLOCK C

Exploiting the Upc2 regulon to overcome triazole-resistant *C. auris*

Laura Doorley, Sarah Jones, Jeffrey Rybak

St. Jude Children's Research Hospital, USA

Abstract

Background: Previously, we have shown loss of the *Candidozyma auris* transcription factor Upc2 transforms the activity of triazoles from fungistatic to rapidly fungicidal even against triazole resistant isolates. Here we identify key Upc2 transcriptional targets and demonstrate they can be exploited as triazole co-therapeutic targets for treatment of *C. auris* infections.

Methods: Transcriptional profiling of SKU067 (triazole resistant) and the derived SKU067-*upc2*^{dis} was performed with and without posaconazole treatment. Minimum inhibitory concentrations for triazoles, abafungin, fluvastatin, β-amyrin, fenpropimorph and terbinafine were determined by broth microdilution. Fungicidal activity for single and combination treatments was evaluated by time-kill analysis. A neutropenic murine model of invasive candidiasis evaluated treatment *in vivo*

Results: Murine modeling revealed the absence of *UPC2* confers fungicidal activity to posaconazole *in vivo*, dramatically reducing SKU067-*upc2*^{dis} fungal burden (>3 $\log_{10}$ CFU reduction) in blood and all harvested tissues, while having minimal impact against the parental triazole resistant SKU067. RNAseq identified key Upc2 transcriptional targets following posaconazole treatment with *ERG2*, *ERG24*, *ERG6*, *ERG1*, and *HMG1* among the most strongly down-regulated genes in the absence of *UPC2* (6 to 105-fold decreased). Inhibitors of Hmg1 (fluvastatin, β-amyrin), Erg6 (abafungin), Erg2/Erg24 (fenpropimorph), and Erg1 (terbinafine) all exhibited fungistatic effect as monotherapies against SKU067. However, combination of any one of these compounds with therapeutic posaconazole concentrations resulted in fungicidal activity against SKU067

Conclusion: These data demonstrate the therapeutic potential of triazole co-therapeutics targeting key *C. auris* Upc2 transcriptional targets (Erg2, Erg24, Erg6, Erg1, and Hmg1). Further development of these combination therapies is needed.

BLOCK C

Phosphate acquisition and starvation in *Candida albicans*

Wanjun Qi[1], Maikel Acosta-Zaldivar[1,2], Udita Roy[1], Abhishek Mishra[3], Matthew Z Anderson[3], Julia Koehler[1]

[1]Boston Children's Hospital, USA. [2]Planasa, Spain. [3]University of Wisconsin, USA

Abstract

Background. Fungi frequently experience scarcity of inorganic phosphate (Pi), an essential macronutrient. Regulatory systems including Target of Rapamycin Complex 1 (TORC1) respond to Pi availability. A *Candida albicans* Pi transporter was identified through decreased TORC1 signaling of its mutant.

Methods. We generated deletion mutants in each of the 4 Pi transporters identifiable in the *C. albicans* genome. We also created triple mutants in which a single transporter alone remained active, as well as quadruple mutants deleted for all 4 Pi transporters. The contribution of each Pi transporter to growth, Pi uptake, resistance to stressors and TORC1 signaling was analyzed. 4 quadruple mutants lacking all 4 Pi transporters were evolved by serial passage in Pi scarcity for 2 months and their stress phenotypes were assayed. Genotypes of 2 lineages were characterized by whole genome sequencing (WGS).

Results. Pho84 is the most active Pi transporter at acidic and alkaline pH. It is the only transporter required for normal TORC1 signaling. Pi uptake is most efficient at pH3-6, far below pH of human tissues. Lineages evolved distinct stress response trajectories. WGS showed early, similar large-scale genomic rearrangements in 2 lineages. These were later replaced by several small-scale mutations in specific genes, skewed towards those related to TORC1 signaling.

Conclusion. Virulence- and stress defects of *C. albicans* lacking only Pho84 among the 4 Pi transporters, are attributable to its predominant role for uptake of the essential nutrient Pi. In vitro evolution might be used like a forward genetic screen to ask open-ended questions.

BLOCK C

Translational control of *Candida albicans* pathogenicity by eukaryotic translation initiation factor 3 (eIF3)

Madison Allegretti, Sohail Khoshnevis

Emory University, USA

Abstract

During its morphological transition from yeast to the pathogenic hyphal form, *Candida albicans* must turn on a genetic program to express virulence genes related to hyphal growth, biofilm formation and tissue invasion. The expression of these genes is regulated at the transcriptional and post-transcriptional levels to allow rapid cellular response to external cues. In other eukaryotes, control of gene expression at the translational level is a common regulatory pathway, with eukaryotic initiation factor 3 (eIF3) playing a major role by regulating translation of specific mRNAs. In this work, we aim to characterize the eIF3-mediated translational control of *C. albicans* during yeast-to-hyphae transition. We isolated eIF3 from yeast and hyphae forms of *C. albicans* and employed mass spectrometry and RNA-seq methods to assess protein and RNA interactomes, respectively. We found surprising differences in composition and interactome of eIF3 between the two morphological states. These investigations will provide insight to the molecular strategies that *C. albicans* utilizes for pathogenicity as well as reveal possible druggable targets at the translational level for development of novel antifungals.

BLOCK C

Interkingdom communication via extracellular vesicles: indirect interactions between oral pathogenic bacteria and *Candida* species

Zóra Szilovics[1], Éva Veres[1], Krisztina Buzás[2], Attila Gácser[3,4,5]

[1]Department of Biotechnology and Microbiology, University of Szeged, Hungary. [2]Synthetic and System Biology Unit, Hungarian Academy of Sciences, Biological Research Centre (BRC), Szeged, Hungary. [3]HCEMM-USZ Fungal Pathogens Research Group, Department of Microbiology, Szeged, Hungary. [4]HUN-REN SZTE Pathomechanisms of Fungal Infections Research Group, University of Szeged, Hungary. [5]University of Szeged, IKIKK, Competence Centre for Molecular Biology, Bionics and Biotechnology, Hungary

Abstract

The human oral cavity is colonized by more than 700 microbes, such as bacteria, viruses, fungi, known as the oral microbiota. As a result of environmental effects, such as smoking or infections, the microbial composition may change, which can result in dysbiosis that may lead to diseases, such as oral candidiasis. Oral *candidiasis* is most commonly caused by *Candida albicans*, which can alter the bacterial diversity.

To examine the nature of such fungal-bacterial interactions, we aim to investigate the interaction between *Candida* species- and oral pathogenic bacteria at the level of extracellular vesicles (EV).

For our experiments we used the *C. albicans* SC5314 and *C. parapsilosis* CLIB214 strains, along with *Staphylococcus aureus* as pathogenic bacterial counterpart. We optimized the fungal and bacterial EV isolation protocol from solid media. The characterisation of the EVs by transmission electron microscopy and NanoSight showed round shaped particles with diameters between 50 and 250 nm.

We examined the effects of EVs released by *C. parapsilosis* and the yeast and hyphae form of *C. albicans* on the growth and biofilm formation efficiency of *S. aureus* and vica versa. As a results, we found that EVs from *C. albicans* and *C. parapsilosis* had different effects on the growth and biofilm formation efficiency of *S. aureus.*

Regarding the effect of bacteria, the *S. aureus* EV treatment can induce the hyphae formation of *C. albicans* cells. Altogether these results suggest the presence of an active interaction between fungal and bacterial cells at the level of EVs.

BLOCK C

Does *Candida parapsilosis* influence the immune regulation in health and oral carcinoma?

Máté Lajos Csikós[1], Zsolt Tasi[1], Attila Gácser[2,3,4]

[1]Department of Biotechnology and Microbiology, University of Szeged, Hungary. [2]HCEMM-USZ Fungal Pathogens Research Group, Hungary. [3]HUN-REN-USZ Pathomechanisms of Fungal Infections Research Group, Hungary. [4]University of Szeged, IKIKK Competence Centre for Molecular Biology, Bionics and Biotechnology, Hungary

Abstract

Trained immunity is part of the innate immune system. Unlike adaptive immunity, it does not involve antibodies to form immune response, and its memory is enabled by epigenetic and metabolic reprogramming. This phenomenon can be found in professional immune cells as well as in epithelial cells.

Oral cancer is among the most prevalent malignancies worldwide. The most frequent form of oral cancer is squamous cell carcinoma (SCC). Oral fungal infection may be a consequence of antitumor therapy, but fungal overgrowth itself may contribute to carcinogenesis. *Candida albicans* is the most common pathogenic fungus in the cancer microbiome, it is frequently detected in these malignancies.

To study the biological event of trained immunity in health and disease, we made a model system where we stimulated both healthy and OSCC cell lines with *Candida parapsilosis* GA1 and CLIB214 strains for 24 hours. After five day resting period, we exposed the host cells to *Candida albicans* SC5314 and WO-1 strains to assess the impact of prior fungal stimulation. We examined the effect of pre-treatment on cell damage, cytokine production, fungal adhesion capacity and gene expression changes in host cells.

Our results revealed that pre-treatment with *Candida parapsilosis* reduced cytotoxicity, increased the production of pro-inflammatory cytokines IL-6 and IL-8, enhanced the adhesion ability of *C. albicans* strains to the host. These findings suggest that prior exposure to *C. parapsilosis* may induce trained immunity-like response in epithelial cells, influencing host-pathogen interactions and immune modulation in oral cancer.

BLOCK C

Deciphering the antibody response against *Candida* virulence factors

Rosanne Sprute[1], Florian Pley[1], Albert Wahl[1], Androniki Kolovou[1], Sibylle Mellinghoff[1], Bernhard Hube[2], Shakti Singh[3], Ashraf Ibrahim[3], Florian Klein[1], Jan Rybniker[1], Alexander Simonis[1], Oliver A. Cornely[1]

[1]University of Cologne, Germany. [2]Hans Knoell Institute, Germany. [3]Harbor-UCLA Medical Center, USA

Abstract

Introduction:

Candida species express various secreted and surface-associated antigens that mediate adhesion, immune evasion, tissue damage, and nutrient acquisition. Understanding the humoral immune response during invasive candidiasis could inform novel diagnostic and therapeutic strategies.

Methods:

To profile the IgG host response to commensalism and invasive candidiasis, we analysed serum IgG responses in healthy volunteers (n=27) and patients with invasive *Candida* infections (n=53) using ELISA against the recombinantly expressed *Candida albicans* proteins agglutinin-like sequence 3 (Als3), hyphally regulated cell wall protein 1 (Hyr1) and secreted aspartyl proteinases (Sap) 1, 2, 6, and 9.

Results:

All participants showed detectable antibody levels against the tested antigens. Titres were significantly higher in infected patients for Als3, Sap1, Sap2, Sap6, and Sap9 compared to healthy controls, with the most marked difference observed for Als3 ($p<0.001$).The median half-maximal effective dose (ED50) for Als3-reactive antibodies was 1772.7 in patients with invasive candidiasis versus 134.8 in healthy individuals, indicating a 13.2-fold increase post-infection. Hyr1 titres showed no significant difference between the cohorts.

Conclusion:

All individuals carry detectable levels of antibodies against *Candida* antigens, indicating a permanent host-pathogen interaction with commensal *Candida* spp. Invasive infection substantially amplifies this humoral response, particularly against Als3, potentially reflecting a protective immune response.

Outlook:

We plan to isolate antigen-specific single B cells from individuals after invasive *Candida* infection with robust humoral responses. From these cells, we will perform B cell receptor repertoire analysis and subsequent recombinant monoclonal antibody production. Monoclonal antibodies will undergo functional testing to explore their potential in therapeutic use.

BLOCK C

Linking protein turnover and chronological aging by using natural isolates of Saccharomyces cerevisiae

Johanna Ficht[1], Ziyue Wang[1], Christiane Kilian[1], Lisa K. Henning[1], Kathrin Textoris-Taube[1], Daniela Ludwig[1], Michael Muelleder[1], Julia Muenzner[1], Markus Ralser[1,2]

[1]Charité Medical University, Germany. [2]University of Oxford, United Kingdom

Abstract

Maintaining proteostasis is crucial for overall cellular health and highly depends on protein turnover, the delicate balance between protein synthesis and degradation. Dysfunction of protein turnover leads to the accumulation of damaged proteins, which has been associated with neurodegenerative disorders and age-related diseases. Thus, loss of proteostasis has been defined as a significant hallmark of aging. By using the yeast *Saccharomyces cerevisiae* as a model organism, important molecular eukaryotic aging pathways have been identified. However, in recent years, studies revealed that laboratory strains can differ considerably from natural isolates, exhibiting broad genetic variation and differences in phenotypic traits, growth, cell cycle, and reproduction. Previously, we examined the chronological lifespan of four euploid natural isolates under treatments, known to extend lifespan of yeast and higher eukaryotes, i.e. spermidine (SP), and caloric restriction (CR). Surprisingly, the four isolates exhibited a divergent aging behavior with clear differences to the commonly used laboratory strain BY4741. Now, we focus on the naturally occurring polyamine spermidine which is a promising molecule to support healthy aging, but on a proteomic level its lifespan extending effects are still not completely understood. Next, we want to systematically analyze the link between protein turnover and aging. There is strong evidence that SP, and CR impact protein turnover on synthesis level, i.e. translation of specific proteins, as well as degradation level, i.e. autophagy and proteasomal degradation. Thus, our aims are to understand the lifespan extending effect of spermidine and to link protein turnover with chronological aging by using natural yeast isolates.

BLOCK C

Dissecting Translational Ambiguity in *Candida albicans* through a Proteogenomic Approach.

Inês Correia[1], Carla Oliveira[1], Andreia Reis[1], Ana Rita Guimarães[1], Nathiely Manzoni[1], Pedro Domingues[2], Ana Rita Bezerra[1], Manuel A. S. Santos[1,3]

[1]Institute of Biomedicine (iBiMED) and Department of Medical Sciences (DCM), University of Aveiro., Portugal. [2]Department of Chemistry, University of Aveiro., Portugal. [3]Multidisciplinary Institute of Ageing (MIA-Portugal), University of Coimbra., Portugal

Abstract

Candida albicans, a diploid opportunistic fungus, can persist as a benign commensal or transition into a pathogen, particularly in immunocompromised individuals, where it causes infections with high morbidity and mortality. Its adaptability across host environments is supported by traits such as thermal tolerance, morphological switching, and genomic plasticity.

A less-explored contributor to this adaptability is **proteome heterogeneity**, driven by genomic rearrangements, allelic variation, and amino acid misincorporation during translation. Among these, mistranslation—particularly at the ambiguous CUG codon—is of particular interest due to its potential to alter protein function and influence cell behaviour. However, detecting and quantifying mistranslation events across the proteome remains technically challenging.

To address this, we developed an integrated proteogenomic workflow combining high-resolution mass spectrometry with tailored bioinformatics to sensitively detect rare amino acid substitutions on a proteome-wide scale. We applied this to both wild-type *C.albicans* and an engineered strain with increased leucine misincorporation at CUG sites.

Our results show that *C. albicans* misincorporates amino acids at multiple codon sites above background levels, with a particular impact on CUG positions. Notably, increased leucine incorporation at CUG sites did not compromise translational fidelity elsewhere in the genome, suggesting codon-specific flexibility.

These findings highlight translational ambiguity as a previously underappreciated mechanism shaping proteome complexity, thus phenotypic diversity in *C. albicans*. This plasticity may be modulated by environmental conditions and play a role in pathogenesis. Our approach provides a foundation for future studies exploring translational fidelity in clinical isolates and the potential of mistranslation markers as predictors of pathogenicity.

BLOCK C

Candida Genome Database: The Power of Yeast Genetics for a Pathogen

Jodi Lew-Smith, Jon Binkley, Gavin Sherlock

Stanford University, USA

Abstract

The *Candida* Genome Database (CGD, http://www.candidagenome.org/) serves as an online compendium of experimental information about pathogenic *Candida* species, including *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. dubliniensis*, and *C. auris*. CGD maintains the most current reference sequences for genomics, while associating deep literature-based knowledge with each annotated feature. In the past year we have imported the most informative expression datasets for each species, such that transcriptomic results can be quickly clicked through to experimental results. There are currently 15 *C. albicans*,10 *C. auris*, 3 *C. dubliniensis*, 7 *C. glabrata*, and 5 *C. parapsilosis* expression sets, all of which are viewable in multiple track types. To better address the needs of clinicians studying *Candida*, we have incorporated literature topics that allow searches by disease name to find relevant papers annotated in the database. Further, our extensive resources for orthology within both *Candida* species and other well-studied fungi make it possible to search by disease name and then find suggestions for relevant orthologs. To highlight breakthroughs in *Candida* research, we include a Research Spotlight feature in our quarterly newsletter. Recently we introduced a new Public Wiki that puts a wealth of comparative knowledge on *Candida* biology into one assembled source. CGD serves as a hub for meeting announcements, job opportunities, and news about *Candida* research. Reach the CGD staff at candida-curator@lists.stanford.edu, where we welcome all questions or comments.

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BLOCK C

A Targeted CRISPR-Cas9 Screen Identifies Emerging Regulators of Oxidative Stress in *Candida albicans*

Pedro Miramón, Lorenz Michael

UTHealth, USA

Abstract

The *Candida albicans* genome contains a large number of genes with unknown function, many of which are transcriptionally induced during host interaction. To identify new contributors to fungal stress adaptation and immune evasion, we constructed a CRISPR-Cas9-based deletion library targeting over 80 previously uncharacterized genes in the SC5314 background. These genes were selected based on their transcriptional upregulation during confrontation with macrophages.

The mutant library was screened across ~40 in vitro conditions, including oxidative, osmotic, cell wall, and pH stressors, as well as morphology-inducing environments. From this screen, we identified a small set of mutants with reproducible oxidative stress phenotypes. Several displayed sensitivity to hydrogen peroxide, paraquat, or menadione under otherwise permissive conditions, suggesting a role in redox homeostasis.

A subset of these strains is currently being evaluated in macrophage interaction assays to assess intracellular survival, phagosomal pH modulation, and hyphal escape. While further genotypic validation is ongoing, preliminary data suggest that some of these mutants may influence fungal behavior within host immune cells.

These findings highlight the value of phenotype-driven screening focused on macrophage-responsive genes and suggest that previously uncharacterized loci may contribute to oxidative stress resistance and host adaptation in *C. albicans*. Ongoing work is focused on confirming genotype-phenotype relationships and expanding the functional characterization of candidate genes.

BLOCK C

Feedback Regulation Between Cdc14 and the Ndr Kinase Complex Cbk1/Mob2 in Candida albicans

Patricia Rojo[1], Antonio Esperilla-Muñoz[1], Nuria Trevijano-Contador[1], Soledad Píriz-Antúnez[1], Toni Ciudad[1], Encarnación Dueñas[2], Carlos R Vázquez de Aldana[2], Jaime Correa-Bordes[1]

[1]Dpto. Ciencias Biomédicas. Universidad de Extremadura, Spain. [2]Instituto de Biología Funcional y Genómica. USAL-CSIC, Spain

Abstract

Dephosphorylation of Cdk substrates is a hallmark of mitotic exit in eukaryotic cells. In *S. cerevisiae*, the highly conserved Cdc14 phosphatase is essential for downregulating cyclin-dependent kinase activity during mitotic exit. However, this essential role is not broadly conserved. In *C. albicans*, unlike in *S. cerevisiae*, *CDC14* is non-essential and is not sequestered in the nucleolus during the cell cycle. Here, we describe an interplay between Cdc14 and the RAM pathway that overcomes the inhibition of Cdc14 activity imposed by Cdk1. We show that both Cdc14 and the NDR kinase complex Cbk1/Mob2 —the effector of the RAM pathway—are inhibited by Cdk1-mediated phosphorylation. Moreover, using Phos-tag gels and a GFP-based reporter to monitor Cdc14 activity in live cells, we found that Cdc14 promotes its own dephosphorylation and is inactive in RAM pathway mutants. We propose a model in which Cdc14 is activated during mitotic exit through two sequential mechanisms, producing two waves of activation. First, Cdc14 may be activated by a Mob2-dependent mechanism, which is then amplified by Cdc14 itself through an autocatalytic process that stimulates its own activity.

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BLOCK C

EXACERBATION OF ORAL MUCOSITIS CAUSED BY IL-23-DEFICIENCY AND ORAL CANDIDA ALBICANS EXPOSURE

John Dillon, Heather Conti

University of Toledo, USA

Abstract

Targeted head and neck radiotherapy causes oral mucositis in cancer patients, leading to increased susceptibility to infections of the oral mucosa. Oropharyngeal candidiasis (OPC) is an opportunistic infection caused by the commensal fungus *Candida albicans.* High colonization levels of *C. albicans* are known to worsen damage caused by head and neck irradiation. The IL-23-Th17 axis is a central mediator of immunity to OPC, and anti-cytokine biologics targeting IL-23 have come into widespread clinical use for treating various autoimmune conditions. We sought to understand IL-23-deficiency in the setting of HNI and fungal susceptibility, by taking advantage of a mouse model of radiation-induced oral mucositis combined with fungal infection. Mice lacking IL-23 did not show increased signs of injury to the oral mucosa. However, in mice subjected to targeted radiation and exposed to oral *C. albicans*, damage to the oral mucosa was worse, accompanied by substantially increased fungal susceptibility when IL-23 was absent. Susceptibility was related to a lack of antimicrobial peptide production, not the absence of other antifungal effector cells, including neutrophils. Thus, IL-23-driven control of fungal infections is needed to mitigate susceptibility to oral mucositis, which is relevant to the high proportion of individuals who carry *C. albicans* in the mouth. This suggests that patients receiving treatments that target IL-23 may not need to discontinue therapy should they require head and neck irradiation, but that screening for oral *C. albicans* may be useful to help limit the risk of developing oral mucositis and its attendant adverse events.

BLOCK C

Commonly used drugs promote drug tolerance and resistance in *Candida albicans*

Mariella Obermeier[1], M. Alejandra Esparza-Mora[1], Olivia Heese[1], Nir Cohen[1], Sreejith Jayasree Varma[1], Pinkus Tober-Lau[1], Johannes Hartl[2], Florian Kurth[1], Judith Berman[3], Markus Ralser[1,2,4,5]

[1]Charité – Universitätsmedizin Berlin, Germany. [2]Berlin Institute of Health at Charité, Germany. [3]Tel Aviv University, Israel. [4]Max Planck Institute for Molecular Genetics, Germany. [5]University of Oxford, United Kingdom

Abstract

The rising incidence of fungal infections, especially in patients undergoing complex medical treatments, remains a major concern. Despite this, the effect drugs targeting human biological pathways have on fungal cells, and their potential to influence responses to antifungal therapies, is poorly understood. In this study, we systematically selected and tested a shortlist of non-antifungal medications commonly used in conditions associated with increased fungal infection risk. Focusing on the pathogen *Candida albicans*, we investigated how these drugs affect the pathogen´s response to commonly used antifungals. From 119 drugs frequently co-administered with antifungals across 40 high-risk conditions, 34 drugs were found to alter the susceptibility of *C. albicans* against fluconazole and/ or anidulafungin. These interactions included ten drugs that antagonized fluconazole by increasing antifungal resistance or tolerance levels. This antagonism also manifested in in-vivo survival assays with *Galleria mellonella* larvae. For instance, co-administering the drugs loperamide, estradiol or levothyroxine to fluconazole treatment in *C. albicans* infected *G. mellonella* larvae significantly increased mortality compared to mortality in only fluconazole treated larvae. These findings show that medications commonly used by patients at high risk for fungal infections can directly affect antifungal susceptibility of the fungal pathogen and impair the effectiveness of antifungal treatment. It further suggests polypharmacy as an underappreciated factor in the development of antifungal resistance and tolerance.

Integrating Multi-Layer Genomic Analyses and Phenotypic Data to Map Functional Variation in *Candida albicans*

Shira Milo[1], Sofya Rabinovich[1], Nir Cohen[2], Austi Mottola[2], Judith Berman[1]

[1]Tel Aviv University, Israel. [2]charite, Germany

Abstract

Candida albicans is a major fungal pathogen that displays remarkable genomic plasticity and phenotypic diversity across natural isolates. While large-scale sequencing and phenotyping efforts have generated extensive resources, integrating these layers of information to identify functional genetic variation remains a challenge.

Here we present a framework that combines multi-layer genomic analyses with phenotypic data to uncover functional diversity in a collection of ~1,800 *C. albicans* strains. Genomic variation was characterized at several levels, including single-nucleotide polymorphisms, short indels, ploidy, gene content and integrated with phylogenetic analyses to place genetic differences in population context. Phenotypic assays, including stress response and drug sensitivity, were systematically linked to genotype.

Using this approach, we identified strain subsets with extreme phenotypic values and mapped their unique variants onto protein structures, highlighting candidate variants in genes implicated in DNA damage response and stress tolerance. To support functional prioritization, we developed a statistical framework for testing associations between genetic variation, phenotypic data and metadata, enabling genotype-to-phenotype connections to be drawn across multiple layers of diversity.

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