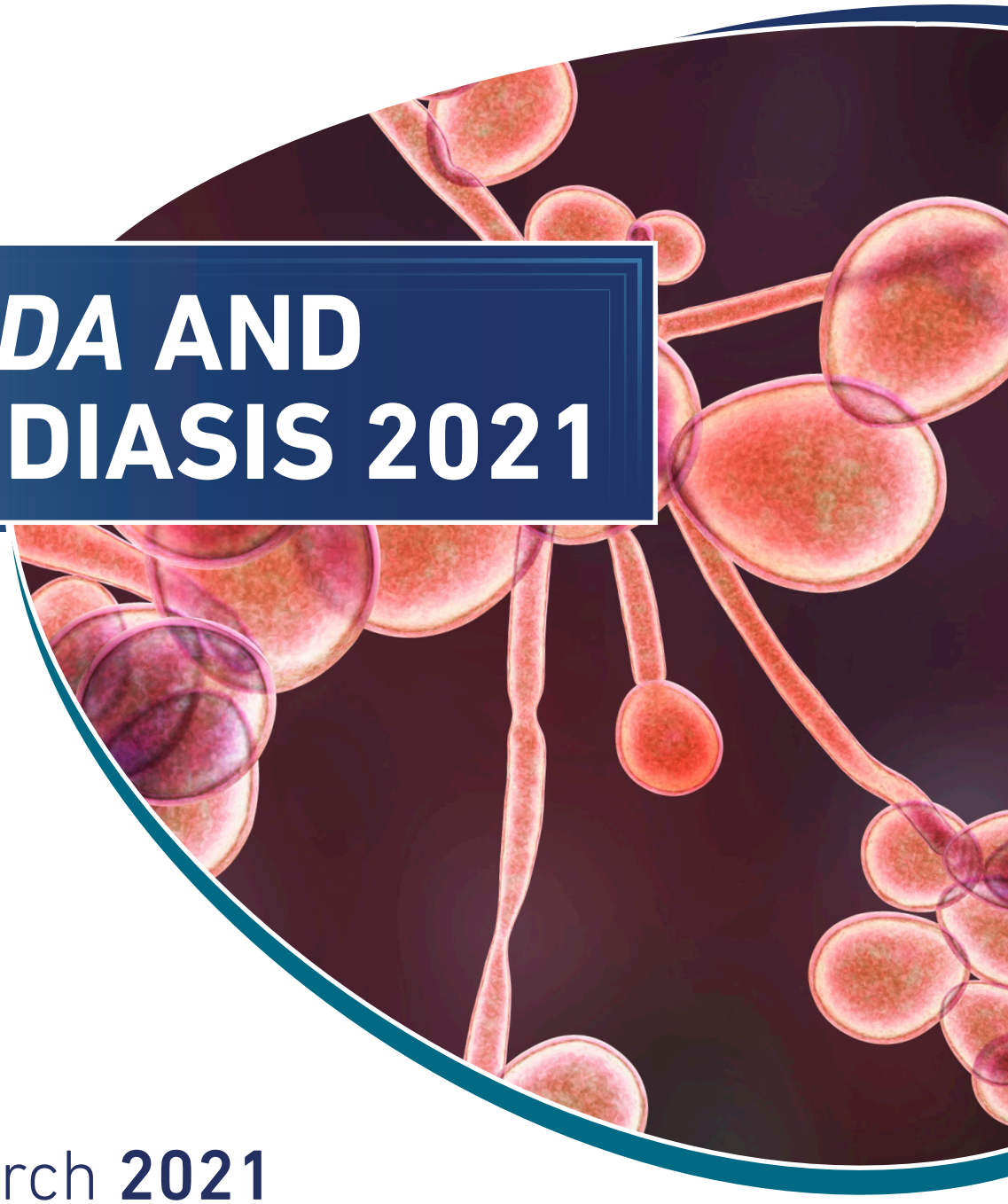
MICROBIOLOGY SOCIETY



CANDIDA AND CANDIDIASIS 2021

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POSTER ABSTRACT BOOK

#Candida2021

001A

Candida auris gene expression: modulation upon caspofungin treatment

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Abstract

Candida auris has emerged as a serious worldwide threat by causing invasive infections in humans that are frequently resistant to one or more conventional antifungal medications, resulting in high mortality rates. Against this backdrop, health warnings around the world have focused efforts on understanding *C. auris* fungal biology and effective treatment approaches to combat this fungus. To date, there is little information about *C. auris* gene expression regulation in response to antifungal treatment. Our integrated analyses focused on the comparative transcriptomics of *C. auris* in the presence and absence of caspofungin as well as a detailed analysis of the yeast's extracellular vesicle (EV)-RNA composition. The results showed that genes coding oxidative stress response, ribosomal proteins, cell wall, and cell cycle were significantly up-regulated in the presence of caspofungin, whereas transcriptional regulators and proteins related to nucleus were down-regulated. The mRNAs in the EVs were associated with the stress responses induced by caspofungin and the ncRNA content of the EVs shifted during caspofungin treatment. Altogether, the results provide further insights into the fungal response to caspofungin and demonstrate that analyses of *C. auris* growth under antifungal stress can elucidate resistance and survival mechanisms of this fungus in response to medical therapy.

002B

Genome-wide analysis of experimentally evolved *Candida auris* reveals multiple novel mechanisms of multidrug-resistance.

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Abstract

Candida auris is globally recognized as an opportunistic fungal pathogen of high concern, due to its extensive multidrug-resistance (MDR). Still, molecular mechanisms of MDR are largely unexplored. This is the first account of genome wide evolution of MDR in *C. auris* obtained through serial *in vitro* exposure to azoles, polyenes and echinocandins. We show the stepwise accumulation of multiple novel mutations in genes known and unknown in antifungal drug resistance, albeit almost all new for *C. auris*. Echinocandin resistance was accompanied by a codon deletion in *FKS1* hot spot 1 and a substitution in *FKS1* 'novel' hot spot 3. Mutations in *ERG3* and *CIS2* further increased the echinocandin MIC. Decreased azole susceptibility was linked to a mutation in transcription factor *TAC1b* and overexpression of the drug efflux pump Cdr1; a segmental duplication of chromosome 1 containing *ERG11*; and a whole chromosome 5 duplication, which contains *TAC1b*. The latter was associated with increased expression of *ERG11, TAC1b* and *CDR2*, but not *CDR1*. The simultaneous emergence of nonsense mutations in *ERG3* and *ERG11* was shown to decrease amphotericin B susceptibility, accompanied with fluconazole cross resistance. A mutation in *MEC3*, a gene mainly known for its role in DNA damage homeostasis, further increased the polyene MIC. Overall, this study shows the alarming potential and diversity for MDR development in *C. auris,* even in a clade until now not associated with MDR (clade II), hereby stressing its clinical importance and the urge for future research.

003C

Comparative molecular and immunoregulatory analysis of extracellular vesicles from *Candida albicans* and *Candida auris*

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Abstract

Candida auris is a recently described multidrug-resistant pathogenic fungus that is increasingly responsible for healthcare associated outbreaks across the world. Bloodstream infections of this fungus cause death in up to 70% of the cases. Aggravating this scenario, C. auris' disease-promoting mechanisms are poorly understood. Fungi release extracellular vesicles (EVs) carrying a broad range of molecules including proteins, lipids, carbohydrates, pigments, and RNA, many of which are virulence factors. Here, we carried out a comparative molecular characterization of C. auris and C. albicans EVs and evaluated their capacity to modulate effector mechanisms of host immune defense. Using proteomics, lipidomics, and transcriptomics, we found that C. auris released EVs with payloads that were strikingly different from EVs released by C. albicans. EVs released by C. auris potentiated the adhesion of this yeast to an epithelial cell monolayer. C. auris EVs also induced the expression of surface activation markers and cytokines by bone marrow-derived dendritic cells. Altogether, our findings show distinct profiles and properties of EVs released by C. auris and by C. albicans, and highlight the potential contribution of C. auris EVs to the pathogenesis of this emerging pathogen.

004A

Comparative phenotypic study of aggregate versus non-aggregate C. auris isolates

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Abstract

Candida auris, first reported in Japan in 2009, has recently emerged on five continents. Due to a particular persistance on a large range of surface types, this *Candida* species has been associated with nosocomial outbreaks.

Candida auris isolates collected during an outbreak in Colombia were identified by MALDI-TOF analysis and ribosomal DNA (rDNA) gene sequencing targeting ITS1 regions. Aggregate and non-aggregate *C. auris* isolates were selectionned for a comparative phenotypic study including sterol composition, cell wall caracterisation, stress resistance, capacity of biofilm formation on catheter pieces and pathogenicity in a *Galleria mellonella* model.

C. auris isolates with aggregating phenotype have a greater capacity to form biofilm. This capacity is even higher if the medium contained glucose as in EUCAST method. In the latter medium, the biofilm formed by this phenotype is superior to that formed by *C. albicans*. Aggregating strains are more sensitive to heat stress while they are more resistant to osmotic shock. In contrast there is no difference in sterol composition. Finally, in the *in vivo Galleria mellonella* model, aggregating phenotype isolates have a lower virulence than those of non-aggregating phenotype.

Our data suggest that using the *Galleria mellonella* infection model**,** non-aggregating *C. auris* strains exhibit higher pathogenicity which could be related to resistance to heat stress.

005B

Molecular epidemiology of *Candida auris* in Qatar using whole genome sequence data

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Abstract

Candida auris is an emerging, multidrug resistant fungal pathogen that has become a public health threat worldwide. *Candida auris* spreads easily among patients within and between hospitals, and the incidence of infections has increased substantially in the last decade. Multiple *C. auris* outbreaks have been reported worldwide including India, USA and United Kingdom. Infections and outbreaks caused by *C. auris* have also been reported in the Middle East region including Kuwait, Oman, Saudi Arabia, and Qatar; however, the origin of these isolates is largely unknown. This study uses whole genome sequencing (WGS) data to determine the epidemiology and the drug resistance mutations from *C. auris* in Qatar. Thirty-five samples isolated from the patients and the hospital environment were sequenced by Illumina Nextseq. Core genome SNPs revealed that all isolates belonged to the Indian lineage, which could be originated from the expatriate labour from South Asia. The genetic variability among the isolates was low but comprised of more than one genetic cluster. The one environmental isolate was identical to the clinical isolates, and the isolates from patients of different hospitals/outbreaks clustered together, suggesting the transmission of *C. auris* could be linked to infected/colonized patients and the hospital environment. Mutations associated with azole and echinocandin resistance were detected.

006C

The effect of acquired triazole resistance on abiotic stress tolerance and virulence in *Candida auris* microevolved strains

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Abstract

Recently, *C. auris* become one of the most prominent members of the genus *Candida*. Since its occurrence, several *C. auris* outbreaks have been reported worldwide. These outbreaks were associated with isolates displaying decreased susceptibility towards fluconazole, the first-line agent for prophylaxis. Fluconazole is the most frequently used antifungal drug to treat bloodstream *Candida* infections.

The physiological effects of acquired antifungal resistance was investigated in this species using fluconazole, posaconazole and voriconazole resistant mutant strains generated by the *in vitro* microevolution method. Alterations in antifungal susceptibility and cross resistance were determined by the microdilution method, utilizing azoles (fluconazole, voriconazole, posaconazole), echinocandins (caspofungin, micafungin, anidulafungin) and a polyene (amphotericin B). Changes in the abiotic stress tolerance was examined by spotting assay, using osmotic stressors, cell wall perturbants and a membrane detergent. To evaluate the impact of the acquired resistance on sterol biosynthesis, ergosterol composition of all generated mutant strains were examined. A potential relationship between virulence and acquired antifungal resistance was also studied both *in vitro* and *in vivo*. Phagocytosis of the generated strains by J774.2 mouse macrophage-like cells was measured and analyzed by flow cytometry. In the murine infection model fungal burden of the triazole evolved strains was determined in spleen, kidney, liver and brain and compared to the fungal burden associated with the initial azole susceptible strain. Significant differences in virulence of the initial and the generated strains was observed suggesting a potential connection between the virulence and antifungal susceptibility of the emerging fungal pathogen, *C. auris.*

007A

Ambisome Shows Potent Anti-*Candida auris* *In vitro* and *In vivo* Activity

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Abstract

Candida auris is an emerging multidrug resistant *Candida* species that has been reported in many parts of the world for causing severe illness in hospitalized patients especially bloodstream infections. The challenge with this type of yeast is its resistance to commonly used antifungal drugs, thus identifying antifungals that are effective against this species is critical.

In this study, we determined the *in vitro* activity of Ambisome against 35 clinical isolates of *C. auris* using minimum inhibitory concentration (MIC) assay as well as the efficacy of Ambisome compared to Amphotericin B and fluconazole using a *C. auris* murine disseminated model.

Antifungal activity of *C. auris* against Ambisome and comparators was assessed using a microdilution method performed according to the Clinical and Laboratory Standard Institute (CLSI) M27-A4 methodology. Mice were immunocompromised and challenged with 3 x 10^7 *C. auris* blastopores in 0.1 ml of normal saline (via the tail vein). Treatment efficacy was assessed by determining reductions in mortality as well as decrease in tissue fungal burden (CFUs).

Ambisome showed lower MIC_{50} and MIC_{90} values (1 and 2 µg/mL, respectively) than the comparators tested. Significant efficacy was observed in the Ambisome 7.5 mg/kg -treated group (100% and 90% survival by day 7- and 14-days post inoculation, respectively). Additionally, Ambisome and fluconazole treated groups showed significant reduction in CFUs in the kidneys *(P*- values of 0.028 and 0.022, respectively) compared to the untreated group.

Our data shows that Ambisome shows significant antifungal activity against *C. auris in vitro* as well as *in vivo*.

008B

Functional Genomics Reveal Determinants of Amphotericin B Resistance in *Candida auris*

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Abstract

Candida auris is an emerging multidrug-resistant human fungal pathogen refractory to treatment by several classes of antifungal drugs. However, molecular mechanisms underlying pronounced multidrug resistance and adhesion traits are poorly understood. AmB is a fungicidal drug that, despite its toxic side effects, remains a drug of choice for the treatment of several drug-resistant fungal infections, including those caused by *C. auris*. In this study, we present data that suggests membrane lipid alterations and chromatin modifications are critical processes that contribute to or cause adaptive amphotericin B (AmB) resistance in *C. auris*. To determine the plausible cause of increased AmB resistance, we performed RNA-seq of *C. auris* AmB-resistant strains (n=3, clade I, MIC ≥2.0 ug/ml) in comparison to a sensitive isolate (n=1, clade I, MIC ≤0.25 ug/ml). We identified a marked enrichment of genes involved in lipid and ergosterol biosynthesis, adhesion, transport proteins as well as chromatin remodeling, especially in AmB-resistant *C. auris* strains, when compared to the AmB-sensitive strains. The transcriptomics data correlates well with the observed reduced lipid membrane permeability of AmB resistant strains compared to the sensitive isolates. All AmB resistant strains carry *ERG11* mutations (Y132F, K142R and K143R). The AmB resistant strains also display hyper resistance to cell wall perturbing agents such as congo red, calcofluor white and caffeine. Additionally, we noticed an increased phosphorylation of Mkc1 MAP kinase in response to AmB in resistant strains compared the sensitive isolate. Collectively, these data provide new insights into the mechanisms of AmB resistance in *C. auris*.

009C

Novel *ERG11* and *TAC1b* mutations associated with azole resistance in *Candida auris*

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Abstract

Candida auris is a novel *Candida* spp. that has spread in all continents causing nosocomial outbreaks of invasive candidiasis. *C. auris* has the ability to develop resistance to all antifungal drug classes. Notably, most *C. auris* isolates are resistant to the azole drug fluconazole, a standard therapy of invasive candidiasis.

Azole resistance in *C. auris* can result from mutations in the azole target gene *ERG11* and/or overexpression of the efflux pump Cdr1. *TAC1* is a transcription factor controlling *CDR1* expression in *C. albicans*. The role of *TAC1* homologs in *C. auris* (*TAC1a* and *TAC1b*) remains to be better defined.

In this study, we compared sequences of *ERG11*, *TAC1a* and *TAC1b* between a fluconazole-susceptible and five fluconazole-resistant *C. auris* isolates of clade IV. Among four of the resistant isolates, we identified a similar genotype with concomitant mutations in *ERG11* (F444L) and *TAC1b* (S611P). The simultaneous deletion of tandemly arranged *TAC1*a/*TAC1*b resulted in a significant decrease of minimal inhibitory concentration (MIC) for fluconazole. Introduction of the *ERG11* and *TAC1b* mutations separately and/or combined in the wild-type azole susceptible isolate resulted in a significant increase of azole resistance with a cumulative effect of the two combined mutations. Interestingly, *CDR1* expression was not significantly affected by *TAC1a/TAC1b* deletion or by the presence of the *TAC1b* S611P mutation, suggesting the existence of Tac1-dependent and Cdr1-independent azole resistance mechanisms.

We demonstrated the role of two previously unreported mutations responsible for azole resistance in *C. auris*, which were a common signature among azole-resistant isolates of clade IV.

010A

Signalling transmission is regulated by component interactions in the *Candida albicans* pheromone response pathway

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Abstract

Saccharomyces cerevisiae cells and *Candida albicans* opaque cells release pheromone to stimulate cells of opposite mating type. Although both organisms share the same orthologous proteins required for the activation of the pathway, the reaction cascade does not function the same way implying the regulation is different. We have used GFP-tagged fusion proteins to investigate the localization of the scaffold protein Cst5, as well as the MAP kinases Cek1 and Cek2, during pheromone response in *C. albicans*. In wild-type cells, pheromone treatment directed Cst5-GFP to surface puncta concentrated at the tips of mating projections. The Gα and β subunits presented distinct relationships with Cst5 revealing different roles in the regulation of the pathway. *ste11Δ/Δ* expressed Cst5-GFP signal concentrated in puncta in the absence of pheromone, but with the puncta distributed around the cell periphery in the absence of mating projections. These puncta were absent from *hst7Δ/Δ* cells, but could be detected in the *ste11Δ/Δ hst7Δ/Δ* double mutant. Cek2-GFP showed a strong nuclear localization late in the response, consistent with a role in adaptation, while Cek1-GFP showed a weaker, but early increase in nuclear localization after pheromone treatment. Activation loop phosphorylation of both Cek1 and Cek2 required the presence of Ste11. In contrast to Cek2-GFP, which showed no localization in *ste11Δ/Δ* cells, Cek1-GFP showed enhanced nuclear localization that was pheromone independent in the *ste11Δ/Δ* mutant. The results are consistent with CaSte11 facilitating Hst7-mediated MAP kinase phosphorylation and also playing a potentially critical role in both MAP kinase and Cst5 scaffold localization.

011B

Discovering the chlamydospore regulatory network in *Candida albicans*

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Abstract

A normal resident of healthy humans and warm-blooded animals, *C. albicans* is a commensal fungus that is also among the most common opportunistic pathogens of humans. *C. albicans* forms unique morphological structures called chlamydospores, which are large, spherical, thick-walled structures formed at the ends of hyphae that have unknown biological function. My goal is to discover the regulatory network controlling chlamydospore formation in *C. albicans*. By determining this network, we can gain insight into the biological roles of chlamydospores in the *C. albicans* lifestyle, better understand *C. albicans* morphological transitions, and determine the selective advantage (if any) provided by chlamydospores to this pathogenic fungus. To determine this regulatory network, I have screened a library of 211 *C. albicans* transcription factor (TF) homozygous deletion mutants to assay for their abilities to form chlamydospores under standard chlamydospore-inducing growth conditions. I have identified seven TF mutants that fail to produce any chlamydospores and three TF mutants that produce high levels of chlamydospores relative to WT. To characterize the transcriptional changes occurring during chlamydospore formation, I have performed RNA sequencing (RNA-seq) on these identified regulator mutants to uncover the differentially regulated target genes of each chlamydospore regulator. I will use genome-wide chromatin immunoprecipitation followed by sequencing (ChIP-seq) on epitope-tagged versions of these regulators to determine which genes are directly under the control of each TF. RNA-seq coupled with ChIP-seq will allow me to determine the regulatory network controlling chlamydospore formation in *C. albicans*.

012C

The β-glucan/glycogen complex: a new structural model of the *C. albicans* cell wall

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Abstract

The *Candida albicans* cell wall serves as the interface between the organism and its environment. The *C. albicans* cell wall is primarily composed of complex carbohydrates, *i.e.* glucan, mannan and chitin. β-Glucan is a (1→3, 1→6)-b linked glucopyranosyl pathogen associated molecular pattern which plays a key role in fungal structural integrity and immune recognition. β-Glucan is not typically found intracellularly. Glycogen is a (1→4, 1→6)-a linked glucan that is an intracellular energy storage carbohydrate. We observed that glycogen was routinely co-extracted during the isolation of β-glucan from *C. albicans*. We hypothesized that glycogen and glucan may form a macromolecular complex that links the *C. albicans* intracellular glycogen with the cell wall β-glucan. To test this hypothesis, we examined the glucan/glycogen extracts by NMR to ascertain if glycogen and β-glucan were interconnected. ^{1}H and 2D COSY NMR analyses confirmed the presence of glycogen and β-glucan in the macromolecular complex. Diffusion Ordered Spectroscopy (DOSY) confirmed that the β-glucan and glycogen co-migrated, which indicates a linkage or complex between the two molecules. We have determined that the linkage is not via peptides and/or small proteins because peptidase treatment of the glucan/glycogen complex did not abolish the co-migration. These results demonstrate that glucan/glycogen form a novel complex in *C. albicans*. This unique structure may necessitate a revision of the existing model of the *C. albicans* cell wall to include membrane spanning complex carbohydrates.

013A

Role of SAGA complex subunits in gene regulation of *Candida albicans*

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Abstract

The SAGA (Spt-Ada-Gcn5-acetyltransferase) is an evolutionary conserved multidomain co-activator complex involved in gene regulation through its histone acetyltransferase (HAT) and deubiquitinase (DUB) functions. It is well studied in *Saccharomyces cerevisiae*, and recent reports from humans and *Drosophila* expand its importance from gene transcription regulation to transcription elongation, protein stability and telomere maintenance. In *Candida albicans*, little is known about the components of the SAGA complex and their influence in morphogenesis and stress response. In this work, we analysed individual components of the SAGA complex, their role in morphogenesis and responses to different signalling cues. We initially analysed conditionally repressed strains of SAGA complex subunits involved in the HAT function of the complex: Tra1, Ngg1, Spt7, Spt8, Taf5, Taf6, Taf9, and Taf10. It appears that the Tra1 might be essential for the viability of *C. albicans*, as we failed to obtain homozygous deletions although it showed detectible growth in the conditionally repressed strain. Also, we observed that TBP-associated factors are essential in *C.albicans*, possibly due to their role in the transcription initiation factor TFIID instead of SAGA. We also detected that the Spt8 repressed mutant was extensively invasive in YPD at 30^0C while a repressed Ngg1 was considerably less invasive compared to its wild type. Also, we have seen that the mutations affecting TBP-binding ability confer susceptibility to drugs, temperature, osmotic, oxidative and DNA damage stress. Further, it seems that the modules of SAGA complex might have antagonistic roles in expression regulation but this needs more in-depth study.

014B

Monitoring intracellular heme trafficking with ratiometric fluorescent sensors

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Abstract

To overcome iron limitation in the host environment, C. albicans is able to extract iron from heme and hemoglobin, the largest iron pool in the human body, using a system that involves an extracellular cascade of soluble and cell surface-anchored hemophores, followed by endocytosis of the heme into the cell. An additional, low-affinity pathway can also mediate internalization of free heme. Furthermore, we find that in addition to sources of iron, external hemin and hemoglobin can also be used as sources of cellular heme. However, little is known about the molecules and mechanisms that distribute heme to hemoproteins residing in virtually every cellular compartment – whether synthesized de novo or absorbed from the environment. In order to achieve a comprehensive description of the C. albicans heme internalization and trafficking pathways, we adapted novel intracellular fluorescent heme sensors for C. albicans. We find that in iron-replete cells, significant amounts of labile heme are detected in the cytoplasm. In iron-starved cells in contrast, cytoplasmic labile heme levels are low. External supply of free heme to starved cells results in a rapid influx of heme to the cytoplasm. Supply of heme as hemoglobin, which requires it to transit via the hemophore cascade to the endocytic pathway and the vacuole, leads to much slower increase of labile heme levels in the cytoplasm. These results indicate that the high-affinity extracellular hemophore-dependent pathway and low-affinity free heme uptake pathway are intracellularly distinct as well.

015C

Exploring the role of protein homeostasis in regulating Candida albicans morphogenesis

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Abstract

Protein homeostasis is critical for proliferation and viability of all organisms. For *Candida albicans,* protein homeostasis modulates the yeast-to-filament transition, an important virulence trait. A key regulator of morphogenesis is the molecular chaperone Hsp90, which mediates proteostasis under diverse conditions such that inhibition of Hsp90 induces filamentation. We explored the effect of perturbation of another facet of protein homeostasis and discovered that morphogenesis is also regulated by the proteasome, a protein complex that controls degradation of intracellular proteins. We identified a conserved role of the proteasome in morphogenesis, as pharmacological inhibition of the proteasome induced morphogenesis of *C. albicans* and several related *Candida* species. For *C. albicans,* transcriptional repression of any of 29 of the 33 proteasome subunits induced filamentation. Filaments induced by inhibition of either the proteasome or Hsp90 shared structural characteristics, including aberrant nuclear content, and shared genetic requirements, such as cyclic AMP-protein kinase A signaling. Consistent with a connection between these facets of protein homeostasis, we observed proteasome inhibition results in an accumulation of ubiquitinated proteins that overwhelm Hsp90 function, relieving Hsp90-mediated repression of morphogenesis. To further elucidate the circuitry through which proteostasis regulates morphogenesis, we performed a functional genomic screen to identify genes required for morphogenesis upon pharmacological inhibition of Hsp90. We uncovered mitochondrial dysregulation reduced susceptibility to Hsp90 inhibitors through upregulation of efflux pumps, necessitating increased dosage of inhibitors for filamentation to occur. Together, our findings suggest interconnected facets of proteostasis regulate *C. albicans* morphogenesis and highlight the importance of mitochondrial function in xenobiotic susceptibility.

016A

Automated Quantification of *Candida albicans* Biofilm-Related Phenotypes Reveals Additive Contributions to Biofilm Production

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Abstract

Background: Fungal biofilms promote community persistence, the seeding of new biofilms, and recalcitrance to drugs and immune surveillance. In *C. albicans*, biofilms are built sequentially through adherence of yeast cells to a surface, filamentous invasion of the substrate, formation of aerial hyphae, deposition of extracellular matrix, and the release of disperser cells. Although defects in this process can lead to biofilm fragmentation, the relative importance of filamentation, adhesion, and invasion (FAI) to total biofilm formation remains unclear.

Methods: Current methods to quantify FAI are highly qualitative and often subjective. Here, we designed an informatics pipeline for FAI quantification from solid agar plate images capable of measuring and scoring hundreds of thousands of individual colonies in hours. This method was applied to a collection of 23 clinical isolates to determine the importance and relationship of FAI to biofilm production.

Results: Characterization of 23 *C. albicans* clinical isolates across three media and two temperatures found that some biofilm-related phenotypes were environmentally regulated while others yielded strain-specific effects, highlighting the contributions of underlying genetic diversity to biofilm-related phenotypes. Importantly, the extent of biofilm formation correlated significantly with the additive score for its component phenotypes under some conditions. In addition, the genome reference strain SC5314 was a clear outlier for multiple phenotypes among these strains.

Conclusion: Taken together, development of a high-throughput, unbiased approach to quantifying biofilm-related phenotypes can facilitate genetic dissection of these critical processes to host disease. Additionally, molecular determinants of biofilm-associated processes in SC5314 may not be applicable broadly across *C. albicans* isolates.

017B

An Intersection Between Iron Availability and *Candida albicans* Contact-Dependent Filamentation

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Abstract

Candida albicans invasive filamentation is a key factor in its pathogenesis. It has been shown that the *DFI1* gene is necessary for filamentation in response to contact with a semi-solid surface. An integral membrane protein, Dfi1's ability to regulate filamentation occurs via activation of a downstream MAPK signaling cascade. Identification of downstream targets of the Dfi1 pathway revealed several genes that are also regulated by the transcription factor Sef1. In low iron, Sef1 regulates genes responsible for iron uptake and utilization. This led us to hypothesize that contact-dependent filamentation via Dfi1 can be influenced by Sef1 and the availability of iron. While we showed that Sef1 is not necessary for contact-dependent filamentation, constitutive activation of Sef1 rescues the *dfi1* null filamentation defect. To investigate the ability of Sef1 to overcome the *dfi1* null filamentation defect in its native conditions, we embedded WT and *dfi1* null cells in iron-depleted minimal agarose medium supplemented with various conditions of Ferrous Ammonium Sulfate. After 4 days of growth at 25^{o}C, *dfi1* null cells grown in 20 uM iron were able to overcome the filamentation defect observed in these cells in higher (50-500 uM) concentrations; WT cells were able to filament at all concentrations. RT-qPCR analysis of the gene *CFL5* – a hypothesized target of the Dfi1 pathway and known target of Sef1 – in cells grown in high and low iron conditions revealed both *DFI1*- and iron-dependent expression. This data suggests that a collection of genes is regulated by both contact-dependent filamentation and iron availability.

018C

Elucidating the roles of force-producing motors in *Candida albicans* mitosis: possible mechanisms for generating aneuploidy

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Abstract

Assembly of a bipolar mitotic spindle is essential for equal division of chromosomes in a dividing parental cell. In order to form a bipolar spindle, most eukaryotes require poleward pushing forces from kinesin-5 motors to counterbalance the inward pulling forces from kinesin-14 or dynein. Inactivation of kinesin-5 disrupts this force balance, resulting in chromosome segregation failure. Remarkably, spindle bipolarity and chromosome segregation can be restored when both kinesin-5 and kinesin-14 are inactivated. Here we show that *Candida albicans* does not require kinesin-5, Kip1, for bipolar spindle formation and nuclear division, and that simultaneous loss of kinesin-5 and -14 activity is lethal. However, Kip1-deficient cells often exhibit spindles that break apart and reform two bipolar spindles in a single nucleus. These spindles then separate, dividing the nucleus, and then elongate simultaneously, resulting in multinucleate cells that resemble cells treated with fluconazole. Interesting, Kip1 null strains exhibit enhanced resistance to fluconazole exposure. This data suggests that kinesin-5-independent mechanisms drive spindle assembly and elongation in *C. albicans*, and that *C. albicans* may modulate kinesin-5 activity to generate genetic diversity for survival during stress exposure.

019A

Functional rewiring of Shugoshin in *Candida albicans*

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Abstract

Candida albicans, an opportunistic human fungal pathogen is known to generate aneuploidy to acquire drug resistance. However, accurate chromosome segregation is essential for survival of this pathogen. In this context, we sought out to investigate the functions of Shugoshin, with conserved yet diverse functions across organisms, in *C. albicans*.

In this study, we have explored various functions of Shugoshin (*SGO1*), a yet uncharacterized gene, in *C. albicans*. We show that CaSgo1 retains the conserved functions of ensuring accurate chromosome segregation through promoting correct chromosome biorientation, sensing of microtubule-based tension between the sister kinetochores and maintenance of key cell cycle proteins condensin and Aurora B kinase (Ipl1), at the centromeres. Interestingly, we reveal novel roles of Sgo1 in *C. albicans* that include demonstrating this protein to be a direct regulatory component of the spindle assembly checkpoint complex. Additionally, hitherto unknown, an in vivo association of CaSgo1 with the microtubule spindle has been observed which appears to drive timely spindle dis-assembly following chromosome segregation. These findings indicate a functional rewiring of the crucial cell cycle protein shugoshin in *C. albicans.*

020B

Sky2 protein kinase regulates dipeptide transport in the fungal pathogen *Candida albicans*

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Abstract

Candida albicans genome comprises of 108 predicted protein kinases, many of which are involved in control of cellular processes such as metabolism, morphogenesis and stress responses. However, the exact role of 59 of those kinases remains unknown. To gain insight into their cellular function we generated a comprehensive protein kinase mutant library. Phenotypic MicroArrays were used to examine the metabolic activity of selected uncharacterized kinase mutants under 850 different nutrient conditions. For example, a strain lacking the *SKY2* gene showed a prominent growth defect on dipeptides as a nitrogen source. *C. albicans* Sky2 (CaSky2), together with Sky1 (CaSky1), are orthologues of *Saccharomyces cerevisiae* Sky1, the sole SR-like protein kinase involved in regulation of mRNA metabolism and cation homeostasis. Subsequent analysis revealed no overlap in CaSky1 and CaSky2 function, with CaSky1 being largely dispensable for cellular activity. On the contrary, CaSky2 seemed to play an important role in metabolism of dipeptides. To identify the potential targets of CaSky2, we performed a phosphoproteome analysis and identified more than 30 proteins phosphorylated in CaSky2-dependent fashion, such as the dipeptide transporter Ptr22. Further, the antibiotics NikkomycinZ and PolyoxinD that have dipeptide-like structure did not affect the growth of a *ptr22*Δ and *sky2*Δ mutant, while the growth of the wild-type was severely inhibited. Moreover, overexpression of *PTR22* in *sky2*Δ background rescued the phenotype. Taken together, we show that, compared to ScSky1, CaSky2 has diverged in function to regulate utilization of dipeptides, an abundant nutrient in multiple host niches.

021C

“Tell me what type of extracellular vesicles you secrete, and I will tell you who you are: yeast or hypha”

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Abstract

The transition between yeast and hyphal morphologies plays a crucial role in the pathogenicity of *Candida albicans*. Recent studies have pointed out the great relevance of extracellular vesicles (EVs) secreted by microorganisms in a wide variety of biological processes including interaction with the host. Therefore, the main objective of this work was to compare the EVs secreted by yeast and hyphal forms to shed light on *C. albicans*-host interaction.

EVs were obtained by ultracentrifugation of the culture medium supernatant and analysed by mass spectrometry. They were characterized by transmission electronic microscopy (TEM) and dynamic light scattering (DLS).

DLS and TEM analysis showed that yeast EVs were significantly bigger than hyphal EVs, being most of them in the range between 400 to 500nm while hyphal EVs were ranged mostly around 100-200nm.

Proteomic analysis showed greater protein diversity in hyphal EVs when compared to yeast EVs (up to 1700 different proteins identified versus 300), although less amount of total protein was obtained. Gene Ontology (GO) analysis showed that yeast EVs were enriched in surface proteins while hyphal EVs, although containing also most of these surface proteins, were also significantly and exclusively enriched in proteins involved in protein metabolism (ribosomal proteins, many aminoacid-pathway enzymes and proteasome) and cellular transport. The differences between YEVs and HEVs also prompted a different immune host response, as tested with macrophage cell cultures and human sera from patients with invasive candidiasis.

All these differences point out a possible different biogenesis and roles of EVs secreted by both morphologies.

022A

Extending the proteomic characterization of *Candida albicans* exposed to stress and apoptotic inducers through data-independent acquisition mass espectrometry

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Abstract

Candida albicans is a commensal fungus that causes systemic infections in immunosuppressed patients. In order to deal with the changing environment during commensalism or infection, *C. albicans* must reprogram its proteome. Characterizing the stress-induced changes in the proteome that *C. albicans* uses to survive should be very useful in the development of new antifungal drugs. We studied the *C. albicans* global proteome after exposure to hydrogen peroxide (H_2O_2) and acetic acid (AA), using a DIA-MS strategy. More than 2000 *C. albicans* proteins were quantified using an ion library previously constructed using DDA-MS. *C. albicans* responded to treatment with H_2O_2 with an increase in the abundance of many proteins involved in the oxidative stress response, protein folding and proteasome-dependent catabolism, which led to an increased proteasome activity. The data revealed a previously unknown key role for Prn1, a protein similar to pirins, in the oxidative stress response. Treatment with AA resulted in a general decrease in the abundance of proteins involved in amino acid biosynthesis, protein folding, and rRNA processing. Almost all proteasome proteins declined, as did proteasome activity. Apoptosis was observed after treatment with H_2O_2, but not AA. A targeted proteomic study of 32 proteins related to apoptosis in yeast supported the results found by DIA-MS and allowed the creation of an efficient method to quantify relevant proteins after treatment with stressors (H_2O_2, AA, and amphotericin B). This approach also uncovered a main role for Oye32, an oxidoreductase, suggesting this protein as a possible apoptotic marker common to many stressors.

023B

Genetic background alters the role of SIR2 in phenotypic switching

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Abstract

Background: Chromatin structure plays an important role in defining cell state in the model fungal organism *Candida albicans*. The unresolved role of the histone deacetylase Sir2 in regulating phenotypic switching led us to investigate its involvement in *C. albicans* cell state.

Methods: Homozygous deletions of *SIR2* and complemented counterparts were constructed in SC5314- and BWP17-derived backgrounds in all possible mating type-like (*MTL*) locus configurations (*MTL***a**/α, *MTL***a**, *MTL*α). White-to-opaque switching was determined on solid SCD medium at room temperature after 7 days. Additional aspects of mating, such as the development of mating projections and mating efficiency, were performed on solid Spider medium.

Results: Loss of *SIR2* in the BWP17 strain background increased white-to-opaque switching frequencies of *MTL* homozygous strains compared to wild-type strains. Development of mating projections in response to cells of the opposing *MTL* genotype was not affected by *SIR2* genotype in the BWP17 background, unless both opaque strains were *sir2*Δ/Δ. Furthermore, *SIR2* played an important role in mating efficiency in BWP17-derived strains that was dependent on *MTL* genotype. However, multiple lineages of *SIR2* mutants in the SC5314 background did not exhibit increased white-to-opaque switching phenotypes.

Conclusions: Overall, our findings reveal that *SIR2* may play a role in the white-opaque switch and mating competency in BWP17-derived strains, However, this phenotype is not seen in SC5314-derived strains, and indicates that strain background may play a complicating role in analysis of white-opaque switching and other complex phenotypes.

024C

The Rsr1 GTPase mediates apical dominance via Cdc42-Cla4 activity and indirectly facilitates timely actomyosin ring contraction through correct organisation of septin rings

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Abstract

We have previously shown that the small Ras-like GTPase, Rsr1, mediates directional growth responses in *Candida albicans* hyphae, which are important for tissue invasion. Like most tip-growing cell systems, wild-type *C. albicans* hyphae exhibit apical dominance, where establishment of new polarity axes are suppressed within the apical tip region due to its asymmetric inheritance of cytoplasm and organelles. Further investigation of the *rsr1*Δ mutant showed that vacuolar fusion was stalled at the tethering-docking stage, resulting in retention of cytoplasm and mitochondria in subapical cells and driving them into START (G1 to S-phase). Cells were multinucleate and establishment of secondary growth axes increased 3-fold, indicating that subapical cells underwent START earlier than wild-type cells, thereby demonstrating loss of apical dominance in *rsr1*Δ hyphae. In addition, septin rings were disorganised in the *rsr1*Δ, which led to diffuse septal association of the MEN kinase, Dbf2, and aberrant trans-septal nuclear migration due to delayed actinomyosin ring contraction. These *rsr1*Δ phenotypes were phenocopied by deletion of Cla4, but rescued when Rsr1 was localized to internal membranes by mutation of C-terminal palmitoylation site. Our analyses suggest that Rsr1 regulates Cdc42-Cla4 at the plasma-membrane during bud site selection and substrate penetration, but at endomembranes for vacuole fusion and septin ring assembly. Our results also suggest that translocation of Dbf2 from the Spindle Pole Body to the septin rings is the signal linking the arrival of a daughter nucleus in the apical cell with septal closure. Rsr1 therefore regulates the spatial organization of hyphal growth through multiple mechanisms.

025A

Testing the role of a Candida-specific membrane protein in filamentation and virulence

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Abstract

The gene *orf19.2302* is upregulated during filamentous growth in *Candida albicans*. This gene encodes a protein that is predicted to contain several transmembrane domains and localize to the endoplasmic reticulum. Based on sequence similarities to proteins in other fungi, it is a predicted to function as a permease and may be a transporter of cations between the ER and the cytoplasm. We have previously described a role for this gene in influencing cellular adhesion and growth in embedded conditions, although neither deletion nor over-expression of this gene alters the ability to form hyphae. In testing its function in stress responses, in particular elevated divalent cation concentrations, we discovered that this gene plays a role in metal stress resistance, specifically to zinc and copper stress. Since the gene is expressed in hyphae, we extended our analysis to look at the influence of over-expressing *NRG1*, a repressor of filamentous growth, on the functions of *orf19.2302* in metal stresses and morphology and have seen differences compared to our results in a wild-type strain. We are continuing our work to determine the role of this gene in the mechanism of metal stress responses.

027C

Protective role of ESCRT machinery and lysosomal exocytosis during *Candida albicans* invasion in oral epithelial cells

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Abstract

The mammalian cell has evolved mechanisms to sense membrane tension or damage and respond as needed. In the case of the cell surface, the plasma membrane acts as a barrier to microorganisms and is a conduit by which the host interacts with fungi such as *Candida albicans*. The yeast-to-hypha transition of *C. albicans* is associated with a shift from commensalism to pathogenicity. Epithelial cells are invaded by *C. albicans* hyphae via induced endocytosis or active penetration. During invasion, hyphal extension and secretion of the peptide toxin candidalysin can cause membrane invagination and epithelial damage. Here we characterize the membrane composition and integrity of the *C. albicans*-containing "invasion pocket". We show that the invasion pocket is predominantly derived from plasma membrane invagination, and not endomembrane vesicles. Further, epithelial cells defend themselves from hyphal-induced damage by at least two separate repair mechanisms both involving influx of extracellular calcium. Candidalysin, known to induce plasma membrane rupture and calcium-influx, is secreted into the invasion pocket by the growing hypha. However, epithelial cells respond to candidalysin-induced rupture by the calcium-dependent recruitment of apoptosis-linked gene-2 (ALG-2), which initiates endosomal sorting complexes required for transport-III (ESCRT-III)-mediated scission of the damaged membrane region, possibly resulting in shedding of inserted candidalysin. Silencing of ALG-2 curtails the viability of epithelial cells and decreases the shedding of damaged epithelial membrane. In some instances, invasion pockets longer than 20 mm show evidence of membrane repair even in the absence of candidalysin (*ece1*D/D). In these cases, the plasma membrane is repaired via exocytosis of lysosomes, which patch the ruptured membrane. This study contributes to our understanding of both commensal and pathogenic interactions between *C. albicans* and epithelial cells. It offers an explanation as to why moderate invasion is not necessarily associated with epithelial damage, while excessive invasion in association with candidalysin secretion causes exhaustion of the epithelial repair system leading to epithelial cell lysis.

028A

Polyamine-regulated Morphological Transition in *Candida albicans*

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Abstract

Biosynthesis of the naturally occurring polyamines, putrescine, spermidine and spermine, is tightly regulated by a variety of mechanisms in most organisms because intracellular polyamine accumulation can result in cytotoxicity. Although polyamine biosynthesis and its regulation has been widely studied in many systems, our knowledge of this process in the human fungal pathogen *Candida albicans* is limited. A previous study has shown that inhibition of the activity of ornithine decarboxylase (ODC), the rate-limiting enzyme controlling polyamine biosynthesis, also inhibits the yeast-filament transition. In addition, this transition is associated with increased ODC activity, possibly through post-transcriptional/translational regulation. Here, we focused on two co-regulated genes in the polyamine biosynthesis pathway, *SPE1* and *SPE2*, which encode proteins with putative ODC and S-adenosylmethionine (SAM) decarboxylase activities, respectively. Based on previous studies, we hypothesize that *C. albicans SPE1* and *SPE2* are controlled at the translational level. Deletion of *SPE1* mostly abolished filamentation as shown previously, while deletion of *SPE2* causes a defect in hyphal extension. The 5' untranslated region (UTR) of mRNAs plays an important role in the eukaryotic translation initiation process and we have found that deletion of the *SPE1* 5' UTR also mostly abolishes filamentation. Comparative analysis of *SPE1* and *SPE2* transcript and protein levels is ongoing to determine whether polyamines control filamentation directly via a translational mechanism and/or indirectly through a signal transduction pathway(s). Our current work highlights the importance of polyamine regulation in controlling the virulence properties of a major human fungal pathogen in response to environmental stimuli.

029B

Slf1, a Novel RNA-binding Protein, Controls *C. albicans* Morphogenesis and Stress Responses

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Abstract

The ability of *C. albicans* to undergo a morphological transition from yeast to filamentous cells is critical for biofilm formation, virulence and pathogenicity. In order to survive in the host, *C. albicans* has also evolved an ability to tolerate a variety of environmental stresses. While transcriptional and posttranslational regulatory mechanisms controlling these virulence processes are well-studied, considerably less is known about translational mechanisms. Slf1 is a key La motif RNA-binding protein in *S. cerevisiae* that controls translation of target genes in response to oxidative stress by interaction with polysomes. In order to determine the role of Slf1 in controlling *C. albicans* virulence-related processes, including filamentation and stress responses, we generated a homozygous deletion mutant. The *C. albicans slf1*Δ/Δ mutant showed a significant filamentation defect on both solid Spider medium at 30°C and YEPD + 10% serum medium at 37°C. In addition, this mutant exhibited reduced growth compared to a wild-type (WT) control strain under cell wall stress conditions (Calcofluor white). Interestingly, the *slf1*Δ/Δ mutant also showed enhanced growth compared to the WT control strain in response to amino acid starvation (3-aminotriazole) and oxidative stress (hydrogen peroxide). We are currently investigating the mechanism(s) by which Slf1 may direct translational control of filamentation, stress responses and other virulence-related processes. Our results suggest that RNA-binding proteins play an important role in controlling a wide variety of *C. albicans* virulence properties and pathogenicity.

030C

A screen for chromosome instability identifies a protein required for cell cycle progression in *Candida albicans*

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Abstract

Gene expression homeostasis is critical for cellular growth. The genome of *Candida albicans*, an opportunistic human fungal pathogen, is remarkably plastic with an ability to tolerate copy number variations, single nucleotide polymorphisms, and loss of heterozygosity (LOH) events. Importantly, LOH driven genome instability has serious phenotypic consequences in *C. albicans* such as conferring resistance to antifungals. Using a recently developed genome-wide collection of plasmids, over-expressing more than a thousand *C. albicans* genes, we identified six candidate genes, over-expression of each of which leads to increased LOH in *C. albicans*. One of them, that we named as *HIT2*, codes for an uncharacterized protein with an unknown function. We show Hit2 is essential for viability in *C. albicans* and is constitutively localized at the spindle pole bodies (SPBs), the site of microtubule nucleation. Over-expression of Hit2 causes Mad2-mediated metaphase arrest in *C. albicans*, with unsegregated nuclei and improper mitotic spindle structure. In contrast, Hit2 depletion arrests cells in anaphase, with segregated nuclear masses and an extended mitotic spindle: hallmarks of mitotic exit network (MEN) mutants. The anaphase arrest due to Hit2 depletion is bypassed upon over-expression of a MEN substrate, Sol1, confirming Hit2's role in mitotic exit. Surprisingly, Hit2-like proteins could be found only in a few CTG-Ser1 clade species and the Hit2 protein sequence shows poor homology with any of the known SPB or MEN proteins. Taken together, we show Hit2 as a new member of the SPB-localizing protein family whose cellular levels are critical for both mitotic progression and mitotic exit.

031A

Investigation of the zinc uptake system of the human fungal pathogen ***Candida parapsilosis***

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Abstract

Candida parapsilosis is the second or third most commonly isolated *Candida* species from blood cultures and *is frequently associated with* infections in neonatal intensive care units. *Candida* species have several virulence factors enabling them to adapt to host environmental conditions and cause infections. These factors include adhesion, biofilm formation, and secretion of hydrolytic enzymes, such as acidic proteinases and lipases. *Candida* species also obtain heavy metal ions from their environment, such as zinc. Zinc is a cofactor of several proteins and a vital element in cellular mechanisms of the fungi. On the one hand, the host niche represents a zinc-limited environment, that indirectly inhibits microbial growth. In order to survive in such an environment, these pathogens have evolved a zinc transport system that allows them to access bound zinc ions during infection. On the other hand, high zinc ion concentration within the host can also be toxic to microbes e.g. in the phagosomes of *Mycobacterium tuberculosis* infected macrophages. In case of *C. albicans*, zinc acquisition processes are intensively studied, but we lack information of the zinc uptake, transfer and homeostasis mechanisms in *C. parapsilosis.* Here, predicted potential zinc transporters in *C. parapsilosis* using *in silico* analyses*,* generated homozygous knock out mutants and performed their phenotypical characterization by exposing them to various types of stressors and zinc limiting conditions. Furthermore, we analyzed their virulence traits by examining kinetics of fungal cell uptake by macrophages, their killing efficiency and also investigated zinc ion levels in the phagolysosome during *in vitro* infections.

032B

C. albicans Tor1 N-terminal HEAT repeats are required for modulation of TORC1-activity during stress resistance

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Abstract

C. albicans is exposed to rapidly changing stresses during colonization and infection, imposed by the host immune system or the competing bacterial microbiome. *C. albicans* TOR signaling is predicted to play an important role in stress resistance. Although Tor1 regions that respond to specific stressors have not yet been defined, models of the mammalian Tor (mTor) kinase show that its 20 N-terminal HEAT repeats are exposed at the surface of TORC1 and accessible to interaction with regulatory molecules. We find this N-terminal region of Ca.Tor1 to be essential for TORC1-activity modulation as well as specific stress resistance. Comparing *C. albicans* strains that express either full-length Tor1 or a truncated Tor1 (Tor1-trunc), which lacks 9 N-terminal HEAT repeats, we found defective growth of cells expressing Tor1-trunc during heat-, oxidative- or cell wall stress, but not plasma membrane stress. While abnormally elevated TORC1-activity was detected in Tor1-trunc expressing cells, their oxidative-stress signaling was weak; low oxygen consumption and low intracellular ROS accumulation was also observed under optimum growth conditions. Truncation of the 5'-region increased TOR1 mRNA abundance, likely contributing to increased TORC1-acitivity in Tor1-trunc cells. Our findings highlight the importance of TORC1-activity downregulation in specific stress responses, as well as a potential regulatory role of Tor1 kinase in carbon source catabolism; processes required for *C. albicans* fitness during interaction with the host.

033C

Study of the mechanical effects on the morphogenetic plasticity of Candida *albicans* in vitro using bioreactors.

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Abstract

C. *albicans* phenotypic change depends on the physicochemical properties of the environment. In a pathogenic context, both hyphae and yeasts are respectively involved in invasion and dissemination in the tissues. During tissue exploration, hyphae experience mechanical forces such as compressive stress and friction. Unlike the impact of chemical parameters like pH or nutrients, little is known about how these mechanical aspects affect the transition.

To this end, we have developed microfluidic devices to characterize the impact of compressive stress on yeast-to-hyphal transition. Our bioreactors allowed both live-cell imaging and high throughput experiments to quantify filamentation efficiency. We used a GFP construction on the *HWP1* gene promoter as a reporter of hyphal induction (pHWP1-GFP). The compressed chamber was connected to a second chamber through narrow channels only accessible to hyphae to study mechanical relaxation.

Using these devices, we observed hyphal-specific gene induction under compressive stress in the order of the turgor pressure. The transition efficacy depended on both compressive stress and pH: while we only observed a few percent of induction at pH = 4.5, it rose to about 40% at pH = 5.5 and above. The observation of hyphal growth in the second uncompressed chamber showed that the majority of hyphae produced lateral yeasts while maintaining polarized tip growth.

Together, our results call for a better apprehension of how mechanical stress and chemical cues couple to drive the yeast-to-hyphal transition. This investigation opens new avenues on the understanding of the dynamics of C. *albicans* phenotypic plasticity.

034A

Marker-less CRISPR/Cas9 Mediated Genome Editing in *Candida auris*

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Abstract

Candida auris is a multidrug resistant fungal pathogen that has emerged worldwide. It can cause life-threatening infections in humans, with a mortality rate upwards of 50%. Nearly all clinical isolates have been found to be resistant to at least one class of antifungal agents. The molecular mechanisms underlying its multidrug resistance and pathogenic properties are unknown. Currently, there are few methods for genome editing in *C. auris*, all of which rely on selectable markers that limit the number of modifications that can be made. Here we present a CRISPR/Cas9-mediated genome editing system, adapted from an existing system in *Candida albicans*, to create a recyclable, marker-less genome editing tool in *C. auris*. Using this system, we can successfully delete and reconstitute genes of interest at their native loci. This system enables precise genome editing that can be performed in a high-throughput manner without leaving behind selectable markers in the genome. Development of a system for facile gene editing is a major step towards improving our understanding of this emerging fungal pathogen.

036C

ChIP-SICAP: A New Tool to Explore Gene-Regulatory Networks in Candida albicans and other Yeasts

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Abstract

Chromatin immunoprecipitation followed by mass spectrometry (ChIP-MS) is a powerful method to identify protein interactions and has long been used to gain insights into regulatory networks in relevant fungal species as well as many other organisms. One of the major limitations of classical ChIP-MS approaches is that the technique enriches both soluble protein-protein complexes and chromatin-protein complexes. Therefore, it is not possible to distinguish if interactions have occurred at regulatory sites of genes or between unbound proteins. A technique that overcomes this limitation is ChIP-SICAP (chromatin immunoprecipitation with selective isolation of chromatin-associated proteins). Here we describe a protocol that allows ChIP-SICAP to be applied to *Candida albicans* and other yeasts for the first time. Notably, the technique design permits stringent washing to remove contaminating proteins and antibodies before subsequent mass spectrometry processing, allows for genome-wide mapping of the bait protein by ChIP-seq after ChIP-SICAP from the same sample through a DNA recovery process, and specifically purifies and identifies proteins associating with chromatin around the bait protein. As proof-of-concept, the protein and gene-network of the Hap5 protein, which is part of the CCAAT-binding transcription factor, was established by ChIP-SICAP confirming its role in iron-response in *C. albicans*. In the future, ChIP-SICAP will provide the yeast genomics research community an additional and specific method to explore the complex dynamics of gene-regulatory networks modulating morphology, metabolism, response to stress, virulence, and infection.

037A

“A novel CRISPRi platform to study the role of essential genes in antifungal drug-resistant Candida albicans isolates”

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Abstract

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

038B

Robust fungal ITS-1 amplicon sequencing and taxonomic annotation pipeline

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Abstract

Amplicon-based next-generation sequencing methods allow us to dissect the microbial composition in multiple environments. In contrast to bacterial 16S rDNA, the common targets of fungal amplicon-based sequencing, the internal transcribed spacer (ITS) regions possess unique features such as varying length. These fungal-specific features require pipeline customization for accurate analysis. Using a mock fungal community of twelve strains with defined input rDNA abundance, we validated an ITS sequencing pipeline based on the DADA2 algorithm. DADA2 enables us to distinguish all twelve input strains, even those whose ITS1 sequences identical at over 99%. By optimizing quality filtering prior to denoising, we were able to retrieve 22% sequences that were falsely discarded by the default filtering strategy implemented in the DADA2 package. With the optimized filtering we simultaneously decreased the proportion of reads with sequences that were unexpected from the mock community from 1.7% to 0.9% and reduced 50% of the non-expected ASVs. Due to the long ITS1 amplicon from species such as *Saccharomyces cerevisiae* or *Candida glabrata*, these species are prone to sequencing bias and quality drop-off. Our optimized quality filtering increased the relative abundance of *S. cerevisiae* and *C. glabrata* ASVs. Finally, we can show that using a BLAST-based algorithm based on the UNITE database achieves a higher reliability in taxonomic annotation compared to using the naïve Bayesian classifier implemented in the DADA2 packages. Taken together, our efforts provide a robust fungal ITS sequencing pipeline for mycobiome analysis.

039C

Genetically Encoded Multimeric (GEM) nanoparticles to study the physical properties of the cytoplasm and nucleus in *Candida albicans*

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Abstract

The interior of a cell is a complex and dense network of proteins, nucleic acids and small molecules that act collectively to coordinate all essential biochemical processes of life. Changes in macromolecular crowding affect the efficiency of biochemical processes by changing the diffusive and interactive behavior. *Candida albicans* is an important opportunistic human fungal pathogen that can cause a range of diseases from irritating mucosal infections to a deadly systemic candidiasis. Extensive research efforts have focused on characterizing transcriptional regulatory networks important for *Candida* morphogenesis as well as pathogenesis; however, little is known about how intracellular physicochemical properties modulate growth and virulence of *C. albicans*. We have recently developed Genetically Encoded Multimeric (GEM) nanoparticles, a powerful tool to study macromolecular crowding in live cells. GEMs self-assemble intracellularly into inert, bright, tracer probes of defined shape and size. Using high-resolution imaging and a suite of single particle tracking tools developed in house, we can rapidly monitor and quantitatively analyze GEMs motion, which depends upon the physicochemical properties of the cell. We have engineered both cytoplasmic- as well as nuclear-targeted GEM nanoparticles in *C. albicans*. We are using these tools to investigate how changes in molecular crowding affect growth, fitness and morphological transitions in *C. albicans*.

040A

FungiDB: integrating genomic data for pathogens and model organisms and providing advanced search capabilities and large-scale data analysis.

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Abstract

FungiDB (https://fungidb.org) is a free, online data mining resource supporting fungi and oomycetes. FungiDB is a component of the Eukaryotic Pathogen, Vector and Host Informatics Resources (VEuPathDB.org), which integrates a diverse array of data for hosts, invertebrate vectors of human pathogens, pathogenic and non-pathogenic species and provides sophisticated data mining tools.

VEuPathDB databases offer a one-stop-shop to enable:

1. ***Browsing*** of genomes and gene record pages in an encyclopedic manner to explore all available information and data
2. ***Searching*** integrated datasets via a unique search strategy system that utilizes a web-based graphical interface to facilitate mining of genomes, functional data (e.g. transcriptomic, proteomic, phenomic and variation data), annotation, and the results of in-house analyses (protein domains, orthology predictions, metabolic pathways, etc.)
3. ***Analysing your own data*** through a private VEuPathDB Galaxy workspace that offers preloaded genomes and sample workflows for RNA-Seq data and variant calling analysis. Users can transfer results to the private My Data Sets workspace, explore the data using the search strategy system and publicly available datasets, and view associated tracks in JBrowse
4. ***Annotating genomes*** through whole genome orthology mapping; the user comments system that captures expert knowledge about phenotypes, relevant PubMed records, etc.; and manual editing of genome models via Apollo, a web-based genomic annotation editing platform
5. ***Learning*** via series of workshops, webinars, and tutorials

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041A

Identification of the substrate-binding sites in the phosphatidylserine synthase from *Candida albicans*

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Abstract

Systemic infections by *Candida spp.* have high mortality rates, and this is related in part to limitations in current antifungals. Thus, there is a need for novel antifungals. The phosphatidylserine (PS) synthase enzyme Cho1 from *C. albicans* has recently been identified as a potential drug target because it is required for virulence in mouse models of Candidiasis, is absent from mammals, and is highly conserved among fungal pathogens. Inhibitors of Cho1 could be used as lead compounds for drug development, but the structure of Cho1 has not been solved and the binding sites for its two substrates, cytidine-diphosphate-diacylglycerol (CDP-DAG) and serine, have not been well-defined. Therefore, we are biochemically addressing this. For CDP-DAG, a conserved CDP-alcohol-binding (CAPT) motif is present in Cho1. We tested this site for its role in PS synthesis by mutating its residues using standard alanine-scanning mutagenesis, and performing growth assays and PS synthesis assays. These experiments revealed that some of the residues within the CAPT motif are involved in Cho1 function. For the serine-binding site, by using computational tools, we predicted a putative serine binding site. Using alanine-scanning mutagenesis, we found that some of these predicted residues are required for Cho1 function. In addition, one of the putative serine binding site residues, R189, affects the Km for serine, indicating that this motif affects serine-binding. We plan to determine if this motif directly binds serine, as this could be useful in future inhibitor development studies.

042B

Influence of arachidonic acid on *Candida albicans* susceptibility to fluconazole

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Abstract

Immunocompromised patients are at great risk of infection by opportunistic fungal pathogens such as *Candida albicans*. Moreover, *C. albicans* has developed resistance to many antifungals. Polyunsaturated fatty acids, such as arachidonic acid, have been reported to increase the susceptibility of *C. albicans* biofilms to azoles. However, the underlying mechanism of action is not fully understood. This was addressed through a combined approach, utilising RNA-seq and phosphoproteomics of *C. albicans* exposed to arachidonic acid and fluconazole. This showed that arachidonic acid physically interacts with cell membranes and may disrupt the movement of glucose and Pi through membranes. Not only are various cellular processes affected, including glycolysis and oxidative phosphorylation, but genes associated with membrane organisation and antifungal resistance are also affected. The pleiotropic drug resistance protein Cdr1p, capable of exporting xenobiotic compounds such fluconazole, is induced upon exposure to arachidonic acid. However, it exhibits a significant impairment of function through the rhodamine 6G assay. This phenomenon may be due to protein mis-localisation, impairment of ATP production and/or phosphorylation. The study not only addresses the impact of arachidonic acid on *C. albicans* growth and metabolism, but also provides a potential explanation for the increased susceptibility of C. albicans exposed to arachidonic acid. Therefore, this study potentiates research into polyunsaturated fatty acids as combination therapy with antifungal agents.

043C

Farnesol abrogates biofilm- and efflux pump- associated genes in drug resistant *Candida auris* strains

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Abstract

Background: *Candida auris*, a decade old *Candida* species, has been identified globally as a significant nosocomial multidrug resistant (MDR) pathogen responsible for causing invasive outbreaks. Biofilms and overexpression of efflux pumps such as Major Facilitator Superfamily and ATP Binding Cassette are known to cause multidrug resistance in *Candida* species, including *C. auris*. Therefore, targeting these factors may prove an effective approach to combat MDR in *C. auris*.

Methods: In this study, 25 clinical isolates of *C. auris* from different hospitals of South Africa were used. Antifungal susceptibility profile of all the isolates against commonly used drugs was determined following CLSI recommended guidelines. Rhodamine-6-G extracellular efflux and intracellular accumulation assays were used to study active drug efflux mechanism. We further studied the role of farnesol in modulating development of biofilms and drug efflux in *C. auris*. Down-regulation of biofilm- and efflux pump- associated genes by farnesol was also investigated. CLSM analysis for examining *C. auris* biofilm architecture among treated and untreated isolates.

Results: Most of the isolates (twenty-two) were found resistant to FLZ whereas five were resistant to AmB. All the isolates were found capable of biofilm formation and ornamented with active drug efflux mechanism. The MIC for planktonic cells ranged from 62.5-125 mM and for sessile cells was 125 mM (0 h and 4 h biofilm) and 500 mM (12 h and 24 h biofilm), CLSM studies also confirmed these findings. Farnesol also blocked efflux pumps and down-regulated biofilm- and efflux pump- associated genes.

Conclusion: Modulation of biofilm- and efflux pump- associated genes by farnesol represent a promising approach in combating *C. auris* infection.

044A

Identifying functional target genes of biofilm formation in *Candida albicans* from dissection of the biofilm transcriptional network

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Abstract

The majority of new *C. albicans* infections arise from the presence of *C. albicans* cells existing in biofilms – resilient, surface-associated, communities of microorganisms with distinct properties compared to planktonic (free-floating) cells. Biofilm infections are highly correlated with implanted medical devices, and current antifungal agents are generally ineffective at treating these recalcitrant infections. We previously discovered the core transcriptional network governing biofilm formation in *C. albicans* consisting of six “master” transcriptional regulators and ~1,000 downstream target genes. Here we present an analysis of the target genes of this network by constructing, screening, and characterizing a priority set of 225 of these target gene mutants for biofilm phenotypes *in vitro*. The mutant strains were grown on the bottom of polystyrene plates, under standard biofilm inducing conditions. Quantification of biofilm formation was performed by optical density and confocal microscopy analyses. In addition, a customizable microfluidic flow device integrated with a time-lapse microscope was used to monitor biofilm formation in real-time under constant temperature, nutrient, and flow conditions, which mimics biofilm growth *in vivo*. Our results reveal new target genes required for biofilm formation, some of which encode for enzymes. Overall, identification of these target genes may provide useful information for the development of novel antifungal agents that specifically target the biofilm mode of growth.

045B

A high-throughput screen for inhibitors of a novel Candida target

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Abstract

Candia albicans is the most commonly isolated invasive fungal species in hospital settings worldwide, causing about 65% of systemic candidiasis infections. These infections still carry a higher mortality than bacterial infections highlighting the need for new, well-tolerated antifungals. Additionally, over the last decade Candida isolates have shown resistance to the common antifungal fluconazole, further underscoring the need for novel antifungal agents. However, the high degree of conservation between fungi and humans at the level of cellular function has confounded antifungal drug development. We previously identified a potential drug target in *C. albicans*, with no human homolog and whose disruption leads to the potentiation of two of the major classes of antifungal drugs. In this study, we established a high-throughput screening assay to identify novel compounds in commercially available libraries, using an engineered strain of *C. albicans* in which inhibition of our target correlates to increased expression of GFP. OD_{600} and GFP expression were measured using a BioTek Synergy H4 plate reader over a period of 30 hours at 15-minute intervals. With a Z' prime value of ≈0.80, 9 hours was established to be the optimal timepoint at which plates are read. A pilot screen of four commercial libraries found seven potential compounds with up to 97% response compared to positive control to move forward in validation assays. Thus, a high-throughput screen for the identification of novel compounds against a new target in *C. albicans* was established and provides an essential tool in moving forward with the fight against invasive candidiasis.

046C

The Impact of Lemongrass, Oregano, and Thyme Essential Oils on Candida albicans' Virulence Factors

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Abstract

Increased systemic infections and growing resistance of *Candida* species in immunosuppressed people have prompted research for additional treatment options. The purpose of this study was to investigate the potential of lemongrass, oregano, and thyme essential oils tested individually, combined, and combined with the antifungal agents fluconazole and caspofungin to kill *Candida albicans* isolates in a controlled laboratory setting. A quantitative design was used to analyze the experimental data collected and investigate risk factors related to age, gender, race, and co-morbidities. Kill rates of lemongrass, oregano, and thyme essential oils individually and combined, kill rates of fluconazole, caspofungin, and the kill rates when the antifungals were each combined with the three essential oils were compared using 117 isolates recovered from bloodstream infections between January 2009 through August 1, 2017. There were statistically significant increases in kill rates when the isolates were exposed to any of the combinations of essential oils tested. Data on the covariates age, gender, race, and co-morbidities were assessed for risk factors related to *Candida albicans* bloodstream infections. The age group 25-34, kidney failure, and solid organ tumor cancer, were all statistically significantly associated with an increased risk for *Candida albicans* bloodstream infections and multiple organ failure was negatively associated with the risk. Through the use of essential oils, the ability to reduce the number of patients becoming infected with life-threatening yeast infections could not only reduce mortality but also reduce the costs associated with serious infections.

047A

High-throughput Chemical Screen Identifies Azole Potentiator that Targets Lipid Homeostasis in the Fungal Pathogen *Candida albicans*

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Abstract

Invasive fungal infections have transitioned from a rare curiosity to a major cause of human mortality. The dependence on azole antifungals for the treatment of systemic mycotic disease has led to the development of widespread resistance. A promising strategy to expand the antifungal target space and overcome resistance is combination therapy, as combining drugs has the potential to confer enhanced efficacy and fungal selectivity, and to slow the evolution of resistance. To this end, we screened ~20,000 compounds from the RIKEN Natural Product Depository against *Candida albicans,* a leading fungal pathogen of humans, in combination with the azole antifungal fluconazole. This screen identified NPD827, a molecule that acts synergistically with fluconazole against both azole-sensitive and -resistant isolates of *C. albicans*. Consistent with the observed synergy, genetic depletion of *ERG11*, which encodes the azole drug target, as well as other genes in the ergosterol and sphingolipid biosynthesis pathways, conferred hypersensitivity to NPD827. Additional biochemical analysis revealed NPD827 results in an accumulation of toxic long chain sphingoid bases, and the depletion of glucosylceramides that have key roles in membrane function and organization. These alterations resulted in reduced lateral mobility of the membrane-associated protein Ras1 as well as inhibited *C. albicans* filamentation. The impact of NPD827 on membrane homeostasis was accompanied by the induction of core stress responses, including lipid droplet formation and calcineurin-dependent signaling. Collectively, this work has identified a promising new antifungal compound that exhibits profound effects on membrane homeostasis and potentiates azole activity against a leading fungal pathogen of humans.

048B

Drug tolerance facilitates the evolution of drug resistance in Candida albicans

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Abstract

Background. For *Candida albicans* and Candidiasis, drug resistance is sometimes due to the pre-existence of genetic polymorphisms that bypass the mode of action of the drug, thus conferring a long-term survival benefit. In other cases, resistance is acquired via the evolution of *de novo* genetic polymorphisms. There is evidence that *C. albicans* possess a drug tolerance response which “buys time” for individuals to evolve beneficial mutations. Our goal here is to characterize this poorly understood epigenetic cytoprotective program at the single cell molecular level.

Methods. We developed a nano-litre droplet based *Candida* single cell sequencing platform capable of transcriptionally profiling several thousand individual cells in an efficient manner. We exploit this platform to profile both untreated and drug exposed (incl. fluconazole, caspofungin and nystatin) populations at early time points post-treatment (tolerance) and late time points (resistance) in order to understand survival trajectories. The profile are compared with the matched sequenced genomes.

Results. We show that untreated *Candida* populations exhibit “bet hedging”, stochastically expressing cytoprotective transcriptional programs, and drug tolerant individuals partition into distinct subpopulations, each with a unique survival strategy involving different transcriptional programs. We observe a burst of chromosomal aberrations at two days post-treatment that differ between survivor subpopulation.

Discussion. Our single cell approach highlights that survivor subpopulations pass through a tolerance phase that involves a multivariate transcriptional response including upregulation of efflux pumps, chaperones and transport mechanisms, and cell wall maintenance. Together this suggests that targeting the tolerance response concomitantly with standard therapies could represent an efficient approach to ablating clinical persistence.

049C

Screening of the Calibr ReFRAME and the Broad Institute Repurposing Hub Libraries to Identify Repositionable Drugs for the Treatment of *Candida auris* Infections

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Abstract

Candida auris has emerged as a formidable opportunistic pathogen, affecting patients in hospitals and other health care facilities. *C. auris* strains are often multidrug resistant and their ability to form biofilms further complicates treatment. New therapies are urgently needed. Here we have screened both the ReFRAME collection (>12,000 compounds) provided to us by California Institute for Biomedical Research, as well as the Drug Repurposing Hub Library (>6,000 unique compounds) provided by the Broad Institute. The ReFRAME library was screened at 5 µM for inhibition of biofilm formation. We were able to identify 100 possible hits inhibiting > 40% of biofilm formation. We performed a dose response for each hit, and 26 were confirmed as having 50% inhibition or more at 20 µM. Twenty-two of these compounds were known to have either anti-bacterial, antifungal, or disinfectant properties while the other four have a variety of current and researched uses. The Repurposing Hub library was screened at 20 µM for inhibition of biofilm formation. We identified 33 compounds leading to > 70% inhibition, of which 31 were subsequently confirmed using dose-response assays. Although a majority of confirmed hits were known antiseptics, antibiotics or antifungals, we were able to identify several potential repositionable compounds. In conclusion, we have screened two of the largest repurposing libraries available. In addition to revealing effective antifungals and antiseptics, our screens have identified several promising repositionable candidates that could be used for treatment of *C. auris* infections.

051B

Investigation of the MFS drug transporter family in Candida parapsilosis using CRISPR-Cas9

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Abstract

The function of specific transporters is a key feature underlying drug resistance in *Candida* species. Drug transporters fall into two main classes – ATP-binding cassette (ABC) transporters, and the major facilitator superfamily (MFS). Some members of the drug/H (+) antiporter family (DHA1) of the MFS superfamily have been shown to function as multidrug transporters. We identified 16 members of the DHA1 family in *C.* parapsilosis, that fall into four main clades. These include MDR1/FLR1, associated with multidrug resistance in *C. albicans* (3 members); TPO4, associated with polyamine transport (1 member); NAG3/4, associated with transport of N-acetyl glucosamine (2 members); TPO2/3, associated with polyamine transport (1 member); and TPO1/FLU1, possibly associated with fluconazole resistance (9 members). We are using CRISPR-based gene editing to explore the function of all of the DHA1 family in *C. parapsilosis*. To date we have edited all 16 individual members of the family by introducing stop codons near the start site of translation (ATG) or deleting the whole gene. In addition, we have generated strains in which all members of the MDR1 family or the TPO1 family or NAG3/4 family have been edited. Phenotype tests show that TPO3 is the sole DHA1-type transporter of spermine and spermidine in *C. parapsilosis*. CPAR2_603010, CPAR2_207540, and CPAR2_301760 all belong to the Mdr1 family, and all are involved in transport of 4-nitroquinoline 1-oxide. However, only CPAR2_603010 confers resistance to fluconazole, voriconazole, and cycloheximide. The function of CPAR2_603010 has been verified by complementing the individual gene edit.

052C

Complex sphingolipids: Vital determinants of Drug susceptibility, membrane integrity and pathogenesis of *Candida glabrata*

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Abstract

Complex Sphingolipids (SLs) are unique to fungi, which apart from being novel drug targets, also appear to act as molecular signals, in diverse biological processes. In this study, we have specifically blocked the key synthesis step of SLs metabolism by disruption of the uncharacterized *CgIPT1* gene, which based on homology with other Candida spp., predicted to mediate the conversion of MIPC to $M(IP)_2C$. We followed fusion based PCR homologous recombination method for *IPT1* deletion by using dominant marker *NAT1*. The knockout was selected on a nourseothricin drug plate and confirmed by gene specific PCR and by checking $M(IP)_2C$ levels. We observed that the specific accumulation of MIPC or lack of $M(IP)_2C$ in *C. glabrata* displayed increased susceptibility to both imidazole's (ketoconazole, miconazole and clotrimazole) and triazoles (fluconazole, itraconazole and posaconazole). RNA Sequencing of *Cgipt1Δ* cells revealed no major impact on of expression levels of common MDR determinants albeit a distinct imbalances in expression of lipid homeostasis genes was evident. The Fluorescence Recovery after Photobleaching (FRAP) experiments confirmed that plasma membrane in *Cgipt1Δ* cells display a reduction in micro-viscosity leading to increase in drug diffusion and susceptibility of *Cgipt1Δ* cells. Interestingly, the *Cgipt1Δ* also exhibit attenuated virulence in a murine model. Together, our data confirms the relevance of $M(IP)_2C$ in governing drug susceptibility and virulence in *C.glabrata*.

053A

Suppressor genetics reveals novel inter-domain crosstalk within the multidrug transporter Mdr1 protein

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Abstract

The Multidrug resistance-1 protein (Mdr1p) of *Candida albicans* is a crucial drug/H^+ antiporter within the Major Facilitator Superfamily of proteins involved in the efflux of a broad spectrum of structurally diverse xenobiotic compounds. As a member of the DHA1 subfamily, Mdr1p consists of 12 transmembrane helices (TMHs), divided equally into two Transmembrane Domains (TMDs). How the pseudo-symmetrically positioned TMHs, and the TMDs they compose, communicate with each other remains poorly characterized. In that direction, the recovery of spontaneous chromosomal mutants that negatively affect the primary mutant phenotype can provide essential inter-domain communication insights. For this purpose, in the current study we have performed a suppressor screen for a critically transport deficient mutant G230A, located within TMH-4 of Mdr1p, predicted towards the intracellular space. The recovered suppressor (P528H), that restores the transport capacity of this initially drug susceptible mutant, map to TMH-12, very close to the extracellular space. Since the mutant and suppressor sites occupy the N-domain and C-domain, respectively, and locate at a pseudo-symmetrical position, these results hint to a novel pattern of crosstalk. Additionally, the recovered suppressor mutation restores wild type phenotypes for all tested xenobiotic substrates except cycloheximide, thus implying substrate selectivity. Furthermore, the molecular modeling and docking results suggest a novel compensatory mechanism which is independent of drug binding. Altogether, the present study is a first attempt to gain insights into the transport mechanism of drug/H^+ antiporter using the suppressor genetics approach.

054B

Exploration of anti *EFG1* locked nucleic acid gapmers to control *Candida albicans* filamentation

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Abstract

Introduction: Antisense oligonucleotides (ASOs) have been successfully utilized to silence gene expression for the treatment of many genetic human diseases, and particularly the locked nucleic acid (LNA) chemical modification is extensively used with this propose. However, LNA-modified ASOs have never been exploited for controlling virulence genes of *Candida*.

EFG1 is an important determinant of virulence that is involved in the switch from yeast to filamentous forms in *C. albicans*. Thus, our main goal was to explore LNA antisense gapmers for controlling *EFG1* gene expression and to block *C. albicans* filamentation.

Methods: A set of five LNA-modified gapmers were designed with different chemical modifications (phosphorothioate backbone (PS) and/or palmitoyl-2'-amino-LNA) and ASO length. The *in vitro* performance of the different ASOs was evaluated on their ability to control *EFG1* gene expression, by qRT-PCR, and to reduce *C. albicans'* filamentation, through filaments' enumeration by microscopy. The *in vivo* therapeutic potential of ASOs was assessed using a *G. mellonella* model of infection, through a survival assay.

Results: *In vitro* results showed that all ASOs were able to reduce the levels of *EFG1* gene expression, consequently reducing the levels of *C. albicans* filamentation around 50%. Interestingly, *in vivo* tests showed that the LNA-modified gapmer with PS backbone and palmitoyl-2'-amino-LNA was more effective at preventing *G. mellonella* infections.

Conclusions: Undeniably, this work promotes the development of a novel approach for the treatment of *Candida* infections based on the delivery of ASOs coupled with LNA chemical modification.

055C

Copy number variations and point mutations of ERG11 conferring azole resistance among an emerging clone of *C. tropicalis* in Asia

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Abstract

Background

Azole resistant *Candida tropicalis* has been emerged in Asia, especially regionally disseminated Clonal Complex 3 (CC3). This study was aimed to compare the genomic structural variations of azole target site, *ERG11*.

Methods

A total of 11 strains were selected, consisting of four CC3 strains, 5 non-CC3 strains, and two reference strains (ATCC 750 and MYA-3404). Antifungal susceptibility testing was performed by using the Sensititre YeastOne panel. Genome sequencing was performed after DNA extraction using on both either Illumina HiSeq 2500 or Illumina NovaSeq platform and on the Nanopore MinION platform. The average read depth were 250x. qRT-PCR of *ERG11* was performed.

Results

Fluconazole MICs of CC3 strains were at least 8-fold higher than those of 2 fluconazole resistant (FR) non-CC3 strains. The rest non-CC3 strains and reference strains were fluconazole susceptible (FS). Total genome size (average 14.7 Mb) and each chromosome size were similar among strains. *ERG11* was on Chromosome 5, which was numbered based on NCBI database. Copy numbers of *ERG11* ranged from 3 to 6 copies in CC3 strains, comparing with only one copy in FR non-CC3 and FS strains. Correspondingly, the average *ERG11* expression was higher in CC3 strains than in FR non-CC3 and FS strains (5.20 vs. 026 vs. 0.54). No aneuploidy of Chr5 was identified. The Y132F and S154F mutations in *ERG11* were identified in all CC3 and one FR non-CC3 strains.

Conclusions

Combined increased copy numbers and mutations of *ERG11* was unique in CC3 *C. tropicalis*, contributing to high-level fluconazole resistance.

056A

Delineation of the *Candida glabrata* Upc2A regulon

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Abstract

Background: Resistance of *C. glabrata* to fluconazole has greatly limited its utility in the treatment of invasive candidiasis. The transcription factor Upc2 regulates the expression of sterol biosynthesis genes in yeast. We have shown that disrupting *UPC2A* in *C. glabrata* greatly increases its susceptibility to fluconazole in both susceptible and resistant clinical isolates. Upc2A and its target genes represent a potential pathway for overcoming fluconazole resistance in this species. We therefore sought to delineate the Upc2A regulon.

Materials/methods: RNA-seq was used to compare gene expression profiles of 1) wt vs *Δupc2A* strains, 2) wt and *Δupc2A* strains exposed to the sterol biosynthesis inhibitors (SBI) fluconazole, terbinafine, and fenpropimorph, and 3) strains engineered to carry a Upc2A activating mutation (G898D) vs wt. Global chromatin Immunoprecipitation (ChIP-seq) was used to identify genes whose promoters were bound by Upc2A in strains carrying wt and activated forms of Upc2A ± fluconazole exposure.

Results: The expression of two genes were lower in the *Δupc2A* strains as compared to their wt parents *(ERG25*, *ERG3*). Sixteen genes were commonly upregulated in response to SBI treatment in a Upc2A-dependent fashion, the promoters of 10 of which were bound by Upc2A. Fifteen genes were upregulated in strains carrying an activating mutation in Upc2, the promoters of 8 of which were bound by Upc2A.

Conclusions: This more comprehensive understanding of the Upc2A regulon in *C. glabrata* may eventually lead to strategies to overcome fluconazole resistance and enhance fluconazole activity against this important fungal pathogen.

057B

Role for the phosphatidylinositol 3-phosphate 5-kinase in antifungal tolerance in *Candida glabrata*

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Abstract

Candida glabrata is an opportunistic fungal pathogen of humans, which is intrinsically less susceptible to widely used azole antifungals, that block ergosterol biosynthesis. The major azole resistance mechanisms include mitochondrial dysfunction and multidrug efflux pump overexpression. In the current study, we have uncovered an essential role for the actin cytoskeletal network reorganization in survival of the azole stress. We demonstrate for the first time that the azole antifungal fluconazole induces remodelling of the actin cytoskeleton in *C. glabrata*, and genetic or chemical perturbation of actin structures results in intracellular sterol accumulation and azole susceptibility. Further, we showed that the vacuolar membrane-resident phosphatidylinositol 3-phosphate 5-kinase (CgFab1) is pivotal to this process, as *CgFAB1* disruption impaired vacuole homeostasis and actin organization. We also showed that the actin depolymerization factor CgCof1 binds to phosphatidylinositol 3,5-bisphosphate (PI(3,5)P2), and CgCof1 distribution along with the actin filament-capping protein CgCap2 is altered upon both *CgFAB1* disruption and fluconazole exposure. Additionally, while the F-actin-stabilizing compound jasplakinolide rescued azole toxicity in cytoskeleton defective-mutants, the actin polymerization inhibitor latrunculin B rendered fluconazole fully and partially fungicidal in azole-susceptible and azole-resistant *C. glabrata* clinical isolates, respectively. These data underscore the essentiality of actin cytoskeleton reorganization for azole stress survival. Lastly, we have also shown a pivotal role of CgFab1 kinase activity regulators, CgFig4, CgVac7 and CgVac14, through genetic analysis, in azole and echinocandin antifungal tolerance. Altogether, I shall present our findings on functions and metabolism of the PI(3,5)P2 lipid in antifungal tolerance and virulence of *C. glabrata* .

059A

The complete functional analysis of *Candida glabrata* drug:H^+ antiporters: players in multidrug resistance and virulence

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Abstract

Background: Infections by the opportunistic pathogen *Candida glabrata* are considered a worldwide problem, given its ability to survive in stressful host niches, form biofilms and resist antifungal drugs.

Methods: The functional analysis of the predicted Drug:H^+ Antiporter (DHA) family members was carried out, regarding their role in multidrug resistance (MDR), resistance towards host immune stress, biofilm formation and virulence, resorting to standard susceptibility testing, radiolabeled transport, crystal violet, macrophage engulfment and *Galleria mellonella* infection assays.

Results: Seven out of the ten DHA transporters were found to confer antifungal drug resistance, CgFlr1, CgFlr2, CgTpo1_2, CgAqr1, CgQdr2, CgTpo1_1 and CgTpo3, the last four being upregulated in azole resistant isolates, highlighting their clinical relevance. Further inspection revealed the ability of five DHA transporters to resist specific host niche associated molecules. Specifically, *C. glabrata* was found to require CgAqr1 and CgDtr1 to resist acetic acid stress, CgTpo1_1 and CgTpo4 to resist the human antimicrobial peptide histatin-5 and CgTpo3 and CgTpo4 to resist polyamine stress. Besides playing a role in the export of such different molecules, four DHA transporters were also found to be necessary for biofilm formation, CgQdr2, CgTpo1_2, CgDtr1 and CgTpo4, while four other are required for full virulence of *C. glabrata* in the *G. mellonella* infection model, namely CgTpo1_1, CgTpo1_2, CgDtr1 and CgTpo4. No role could be found for the Yhk8 DHA transporter.

Conclusions: Altogether, this screening has highlighted the promiscuity of the DHA transporters, that play multiple roles in *C. glabrata*'s infections.

060B

Repurposing Histone Deacetylase Inhibitors (HDACi) to treat Candida glabrata Infections

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Abstract

Candida glabrata accounts for 25% of fungal infections in UK hospitals, second only to *C. albicans*. This number is expected to rise given current treatment challenges due to *C. glabrata's* intrinsic anti-fungal resistance. In an effort to identify novel anti-fungal targets in *C. glabrata*, we used comparative genomics within *Saccharomycotina* yeast to determine which genes are under positive selection in this species. Such genes are predicted to have influenced the adaptation of *C. glabrata* from a free-living microbe to a human pathogen, potentially due to novel functional shift(s) of the proteins they encode.

Our analysis predicts that histone acetylation pathways are under positive selection in *C. glabrata.* Therefore we hypothesised that interfering with histone acetylation levels, using histone deacetylase inhibitors (HDACi) will impact *C. glabrata* virulence. By treating clinically isolated *C. glabrata* strains with broad spectrum HDACis we show they have a reduced capacity to form biofilms, are less well adapted to high salt conditions typically found within a human host, and are reverted to an anti-fungal sensitive state. RNAseq analysis on *C. glabrata* typed strain (ATCC2001) indicates that HDACi treatment alters the *C. glabrata* transcriptional response to anti-fungal treatment, rendering it incapable of combating against these drugs. Furthermore, using an *in vivo* worm model of candidiasis we show that HDACi treatment in conjunction with fluconazole increases the survival rate of individuals with *C. glabrata* infections.

Taken together our data suggest that the health threat posed by *C. glabrata* might be addressed by repurposing HDACi to treat this infection.

061C

Candida glabrata is uniquely susceptible to antifungal toxins produced by *Saccharomyces* yeasts.

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Abstract

The opportunistic pathogen, *Candida glabrata*, presents significant clinical challenges owing to its inherent drug resistance and limited treatment options. “Killer toxins,” naturally produced by “killer” yeasts, are antifungal proteins that can disrupt ion homeostasis resulting in cell lysis and apoptosis. Through testing >10,000 interactions between *Saccharomyces* killer yeasts and type strains of pathogenic fungi, we found that *C. glabrata* is susceptible to multiple killer toxins. To test the extent of this sensitivity, 135 diverse strains and isolates of clinical and environmental *C. glabrata* were challenged with killer toxins. Of all the killer toxins assayed, 100% and 98% were found to be sensitive to the K1 and K2 killer toxins, respectively. Related pathogenic yeast species of the *Nakaseomyces* genus in the “glabrata group” are significantly more resistant to killer toxins than *C. glabrata*. Although *C. glabrata* is uniquely susceptible to K1 and K2, exposure to partially purified killer toxins was able to select for killer toxin resistance that resulted in abhorrent phenotypes, including reduced growth rate, increased sensitivity to cell wall damaging agents and antifungal drugs, and reduced virulence in *Galleria mellonella* larvae. Whole genome sequencing identified the genes involved in resistance, including cell wall structure regulating genes such as *KNH1*, *KRE6*, and *FKS1* that potentially prevent toxin interaction with the cell wall. Therefore, killer toxins are broadly antifungal to *C. glabrata* and resistance is often associated with significant fitness tradeoffs. This work demonstrates that killer toxins show potential as therapeutic precursors for the treatment of drug resistant candidiasis caused by *C. glabrata.*

063B

Metallic nanoparticles display antimicrobial activity against Candida auris biofilms

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Abstract

Candida auris is an emergent multidrug-resistant pathogenic yeast, causing major outbreaks in hospitals and other health care facilities with high mortality rates. Biofilms formed by strains of *C. auris* are resistant to environmental conditions and to sanitizing products and display intrinsic resistance against conventional antifungals. Antimicrobial nanomaterials (nanoantibiotics) exhibit anti-biofilm activity against other *Candida* species; therefore, they could represent an alternative to combat *C. auris* biofilms. We have designed protocols for producing easy-to-synthesize silver nanoparticles (AgNPs) and bismuth nanoparticles (BiNPs) using a fast and economical chemical reduction process developed in our laboratory. We evaluated the antimicrobial activity of the obtained nanoparticles against *C. auris* strains (CDC panel AR#0381 - #0390) under both planktonic and biofilm growing conditions. The synthesized nanoantibiotics are small spheroid nanoparticles (<10 nm) coated with biocompatible polymers. Under planktonic conditions, AgNPs MIC values against the different *C. auris* strains were <1 µg/mL, whereas BiNPs MICs were <4 µg/mL. Both AgNPs and BiNPs inhibited the ability of *C. auris* to form biofilms, with IC_{50} values <4.9 µg/mL for AgNPs and <43.6 µg/mL for BiNPs. AgNPs retained potent activity against preformed *C. auris* biofilms, with IC_{50} values <4 µg/mL, whereas somewhat elevated IC_{50} values as high as 31.6 µg/mL for some strains were detected in the case of BiNPs. In conclusion, we generated easy-to-synthesize nanoparticles that display good antimicrobial activity against planktonic cells and biofilms of *C. auris*. These nanoantibiotics can be used for sanitizing healthcare facilities and for developing future antimicrobial therapies.

064C

In silico screening leads to novel scaffolds with both antifungal and anti-NLRP3 inflammasome activity.

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Abstract

Due to structural similarities that exist between established inhibitors of the NLRP3-inflammasome, sulfonylureas Glyburide and MCC-950, and herbicidal-sulfonylureas, that specifically target fungal acetohydroxyacid synthase (AHAS), we sought to determine the potential for compounds to block both inflammation and inhibit fungal growth.

In silico screening of ~250,000 compounds was used to identify a prioritized list of chemical structures capable of inhibiting both targets. Prioritization of the top 1% of scores identified ~70 compounds with a diverse set of scaffolds for testing in vitro. Selected hits were used to assess anti-inflammatory function in a THP-1 challenge model with LPS+ATP and resulting IC_{50} values were obtained. MIC and hyphal-growth assays were conducted to determine potential antifungal activity using media depleted of branched chain amino acids isoleucine and valine, to confirm on target AHAS inhibition.

Identification of hits that exhibited low micromolar activity for NLRP3 and AHAS inhibition were selected for SAR study. In vitro testing of the analogs along with molecular docking led to increased knowledge for lead optimization of the potential hits.

In silico screening has resulted in IC_{50} (IL-1β release) and MIC_{50} (fungal growth) values with low μM potency against several Candida species. In vivo validation will further confirm the potential of the scaffolds for further synthetic-modification for the rationale design of novel dual-purpose drugs

065A

Link between methylglyoxal metabolism and drug resistance in Candida lusitaniae

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Abstract

Methylglyoxal (MG) is a toxic metabolic byproduct that is significantly elevated in certain disease states, including diabetes, uremia, and inflammation. Several lines of evidence suggest that, in *Candida* species, the transcription factor Mrr1 not only regulates azole resistance, but also regulates expression of genes that encode methylglyoxal reductases which detoxify MG. Using mutants constructed in *C. lusitaniae*, via an expression-free CRISPR-Cas9 system, we show that Mrr1 regulates two highly similar MG reductases and both participate in MG detoxification and growth on MG as a substrate. In addition, we show that MG increases Mrr1-dependent expression of MG reductase genes *MGD1* and *MGD2* as well as *MDR1* in *C. lusitaniae*. We demonstrate that MG induction of *MDR1* expression results in significantly improved growth in sub-lethal concentrations of fluconazole in *C. lusitaniae* and several other *Candida* species and that Mrr1 is necessary for this phenomenon. Finally, we also demonstrate that deletion of *GLO1*, the rate-limiting enzyme in another pathway for MG detoxification, leads to increased endogenous MG that increases *MDR1* expression and fluconazole resistance in *C. lusitaniae*. Fluconazole resistance was assessed via the minimum inhibitory concentration (MIC) assay and growth kinetics. Given the physiological relevance of MG in human disease, we propose that induction of *MDR1* in response to MG is a novel contributor to *in vivo* resistance against azole antifungals in multiple *Candida* species.

066B

Tolerance to fluconazole in Candida albicans is regulated by temperature and aneuploidy

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Abstract

Candida albicans is a prevalent human fungal pathogen. Azoles are the most widely used antifungal drugs. Drug tolerance in bacteria is well defined and thoroughly studied, but in fungi, the definition of drug tolerance and the mechanism that drive it are not well understood. Here, we found that a large proportion of clinical isolates were intrinsically tolerant to fluconazole, and/or could be induced by high temperature (37˚C) to become tolerant (conditionally tolerant). When treated with inhibitory doses of fluconazole, non-tolerant strains became tolerant by forming aneuploids involving different chromosomes, with chromosome R duplication as the most recurrent mechanism. Tolerance determines the ability to grow in the presence of fluconazole and other azoles, in a manner independent of the MIC. Both temperature conditional tolerance and the associated aneuploidy were sensitive to FK506, an inhibitor of calcineurin. Intrinsic and conditional tolerance were also abolished by deletions of genes encoding the calcineurin (*CMP1* and *CNB1*). However, the dependence of tolerance on calcineurin could be bypassed by a different aneuploid chromosome. Thus, fluconazole tolerance in *C. albicans* is regulated by temperature and by aneuploidy and is dependent upon aneuploidy, but this dependence can be bypassed by an additional aneuploidy.

068A

Echinocandin drug resistance vs. tolerance among *Candida auris* clinical isolates

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Abstract

Background

Development of echinocandin resistance has been documented in *Candida auris* isolates recovered from patients treated with these drugs. Recently, we showed that echinocandin pharmacodynamic resistance is conferred by specific mutations in *FKS1*. Antifungal drug tolerance, defined as the ability of some cells to withstand killing at drug concentrations above the minimal inhibitory concentration (MIC), has received substantially less attention. However, a thorough understanding of this phenomenon can aid in treatment optimization and help minimize the emergence of resistant mutants.

Methods

Echinocandin-susceptible (micafungin MIC range 0.03-0.5 mg/l), *FKS1* wild-type *C. auris* clinical isolates, representing all geographic clades, *C. albicans* and *C. glabrata* control strains, were incubated (RPMI-1640; $1x10^7$ cells; 37ºC; 130 rpm; 24 h) in 2-fold increasing concentrations (0.03 to 128 mg/l) of micafungin. Next, aliquots were plated and number of cells that survived calculated. The experiments were performed in biological triplicates, and data analyzed using GraphPad Prism.

Results

C. auris did not present a classical bi-modal killing pattern (MIC and MPC-Mutation Prevention Concentration) characteristic for fungicidal drugs activity against other susceptible *Candida* spp. isolates. Response of *C. auris* to micafungin differed between isolates with variable subpopulations (0.01-10%) that survived at drug concentrations that exceed the MIC. Despite low MIC values, micafungin did not reach MPC for any of the *C. auris* isolates.

Conclusion

Echinocandin drug tolerance is a subpopulation effect and its intensity varies between the *C. auris* isolates. The impact of antifungal tolerance on the long-term efficacy of drug treatment and pharmacodynamic resistance development requires further studies.

069B

Development of Echinocandin Tolerance and Resistance in *Candida glabrata*

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Abstract

Background. Echinocandins are the preferred therapy for *Candida glabrata* but the increasing number of echinocandin resistant strains poses a significant clinical challenge. Although echinocandins are considered fungicidal, a subpopulation of *C. glabrata* cells exhibits tolerance to echinocandin exposure, allowing for resistant mutations to emerge. Mechanisms that underpin tolerance and development of resistance are not well understood.

Methods. To investigate the development of echinocandin tolerance, we used an *in vitro* killing assay to analyze the survival of *C. glabrata* reference strain CBS138 during micafungin and caspofungin exposure. Furthermore, a subpopulation of *C. glabrata* cells remaining viable after 24h of above-MIC echinocandin exposure was isolated by fluorescence-activated cell sorting, followed by single cell RNAseq to examine their transcriptional landscape. As no drug controls, we used *C. glabrata* cells growing in stationary or logarithmic phase. A cloud-based bioinformatics platform (www.basepairtech.com) was used to identify differentially expressed genes.

Results. The analysis of scRNAseq data identified significantly (>2-fold, FDR-adjusted p-value<0.05) 44 downregulated genes and 87 upregulated genes in drug-tolerant cells relative to both no drug controls. Gene Ontology analysis showed that the downregulated genes were enriched for those involved in antioxidant and oxidoreductase activities. A number of upregulated genes were involved in DNA topology and chromatin structure, cell cycle checkpoints, and several genes encoding proteins of unknown function specific to *C. glabrata*.

Conclusions. The transcriptome analysis of *C. glabrata* echinocandin tolerant cells is revealing new insights into the physiological state of drug tolerance. Further studies of tolerance-associated genes are necessary to unravel the mechanisms involved.

070C

Activating mutation in Mrr1 leads to upregulation of multiple putative drug transporters and fluconazole resistance in *Candida parapsilosis*

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Abstract

Background

In *C. albicans*, activating mutations in the transcription factor gene *MRR1* result in overexpression of the drug efflux pump gene *MDR1* and increased fluconazole (FLU) resistance. Previously, we identified a *CpMDR1* overexpressing *C. parapsilosis* isolate with amino acid substitution A854V in CpMrr1p. *CpMDR1* deletion did not significantly change FLU MIC, suggesting the presence of other resistance determinants.

Methods

Sanger sequencing was used to identify mutations in *CpMRR1* and *CpERG11* in a collection of 35 resistant clinical *C. parapsilosis* isolates. Relative expression of selected transporter genes was determined by RTqPCR. A plasmid-based Cas9 system was used to make genetic modifications in *CpMRR1*. FLU MICs were determined by broth microdilution.

Results

Of the 23 FLU resistant clinical isolates with wild-type *CpERG11*, 10 contained a non-synonymous mutation in at least one *CpMRR1* allele. The A854V substitution was observed in 7 of these isolates. All isolates containing *CpMRR1* polymorphisms exhibited increased relative expression of a second *MDR1* homolog (CPAR2_601030) and a *CDR1* homolog (CPAR2_304370). Increased *CpMDR1* expression was only associated with homozygous *CpMRR1* mutation. Individual overexpression of CPAR2_601030 and CPAR2_304370 in a susceptible background decreased FLU susceptibility. Introduction of A854V mutation in a FLU susceptible isolate increased expression of all three drug efflux genes and FLU MIC (128-fold). Correction of CpMRR1 mutation led to decreases in efflux pump gene expression and FLU MIC (64-fold).

Conclusion

Prevalence of *CpMRR1* mutations and upregulation of multiple efflux pumps genes indicates a prominent role for *CpMRR1* in clinical FLU resistance in *C. parapsilosis*.

071A

Cloning of the *Candida glabrata* echinocandin target gene *FKS1* and associated analysis of medically-relevant mutations

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Abstract

Background: Echinocandins are currently first-line agents in the treatment of invasive *Candida glabrata* infections. Fungal associated resistance arises upon mutation of genes (*FKS1* or *FKS2*) that encode for the catalytic subunits of the target enzyme, beta-1,3-glucan synthase. **Methods and Results**: A gap-repair approach was used to clone the coding region of *FKS1* from ATCC2001/CBS138 onto a plasmid carrying the nourseothricin resistance marker and a strong promoter (pCN-PDC1). All *fks1Δ* transformants were initially screened via PCR and susceptibility to FK506 (*FKS2* inhibitor) to ensure proper *FKS1* presence and expression, respectively, from plasmid (p*FKS1*). Additionally, consistent with the presence of a strong promoter, we measured a 6.4-fold increase in *FKS1* gene expression (early log phase) and a 2.0-fold increase in Fks1 protein expression in the *fks1Δ* strain carrying p*FKS1*, compared to wild type strains. Several medically-relevant amino acid alterations (i.e. F625S, S629P, D632E) were incorporated into p*FKS1* through site-directed mutagenesis and expressed in the *fks1Δ* strain. Transformants were screened as above and drug susceptibility assays performed with caspofungin and micafungin. Compared to expression of pFks1-wild type, assays revealed 4-fold increases in minimum inhibitory concentrations (MICs) upon expression of pFks1-F625S and 8- to 16-fold increases in MICs following pFks1-S629P expression. **Conclusion**: These studies have provided the tools to test additional amino acid substitutions and analysis into how specific *FKS1* mutations influence drug resistance within an isogenic strain. Additionally, we are currently manipulating pFks1 for future protein structure (e.g. addition of a 12x His tag) and microscopy experiments.

072B

Molecular and phenotypic analysis of *ERG11* from South African clade isolates of *Candida auris*

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Abstract

Background: Strains of the emerging fungal pathogen *Candida auris* demonstrate elevated rates of resistance to antifungal drugs, including triazoles. Our previous work demonstrated specific mutations in the azole drug target, *ERG11*, directly influenced triazole susceptibility in South American and South Asian isolates. Here, we classify mutations within triazole refractory South African clade isolates. **Methods and Results**: Two *ERG11* mutations (t374c/t376c) which lead to two amino acid alterations (V125A/F126L) were identified in each isolate. The Erg11-V125A/F126L allele was cloned onto a low-copy plasmid (pRS416) and expressed in the model organism *Saccharomyces cerevisiae* through gap-repair. Broth microdilution assays revealed decreases in susceptibilities to both short-tailed triazoles (MICs, fluconazole: 32-64 µg/ml; voriconazole: 0.5-1 µg/ml) and long-tailed triazoles (itraconazole: 8-16 µg/ml; posaconazole: 0.5-8 µg/ml) when compared to expression of wild type alleles. To determine the significance of each amino acid alteration, we performed site-directed mutagenesis to individually dissect and express the V125A and F126L alterations. In preliminary assays, the Erg11-V125A allele demonstrated elevated long-tailed triazole MICs but near wild type short-tailed triazole MICs when expressed in *S. cerevisiae*. Oppositely, expression of Erg11-F126L demonstrated near wild type long-tailed triazole MICs but elevated short-tailed triazole MICs. **Conclusion:** The South African Erg11 allele (V125A/F126L) exhibited reduced triazole susceptibilities when expressed in *S. cerevisiae*. This clade may have evolutionarily acquired both mutations leading to pan-azole resistance. Understanding which Erg11 amino acid substitutions lead to reduced drug susceptibility will help define azole resistance mechanisms in *C. auris* and allow for better treatment practices.

073C

Delineation of the direct impact of mutations in *C. auris ERG11* on clinical fluconazole resistance

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Abstract

Background: *Candida auris* is an emerging pathogen of great clinical concern. 90% of *C. auris* clinical isolates are resistant to fluconazole (FLU), and FLU minimum inhibitory concentration (MIC) are commonly greater than or equal to 256mg/L. While mutations in the gene encoding the target of the triazoles, *ERG11*, are common among fluconazole-resistant clinical isolates, the direct contribution of these mutations to clinical FLU resistance remains unknown.

Methods: A Cas9-mediated transformation system was used to individually introduce *C. auris ERG11* alleles harboring mutations encoding the amino acid substitutions VF125AL, Y132F, and K143R, as well as a wild-type control *ERG11* allele, into the FLU susceptible clinical isolate AR0387 (FLU MIC 1mg/L). Additionally, the K143R encoding mutation in the FLU-resistant clinical isolate AR0390 (FLU MIC 256mg/L) was replaced with the *ERG11* wildtype allele. FLU MIC were determined by broth microdilution.

Results: Introduction of the $ERG11^{VF125AL}$, $ERG11^{Y132F}$, $ERG11^{K143R}$ alleles into AR0387 resulted in elevated FLU MICs to 16, 32, and 16 mg/L; respectively, whereas the wildtype manipulation control allele exhibited no change in FLU MIC (1mg/L). Introduction of the $ERG11^{WT}$ allele into AR0390 resulted in a greatly reduced FLU MIC (from 256 to 32mg/L).

Conclusions: These studies demonstrate that mutations in *C. auris ERG11* significantly contribute to clinical FLU resistance. However, these mutations in *ERG11* alone cannot completely explain the high level of FLU resistance commonly observed among clinical isolates. Further research is needed to identify and characterize additional clinical FLU resistance mechanisms.

074A

In vitro evolution reveals *TAC1B* as a regulator of clinical triazole resistance in *C. auris*

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Abstract

Background: *Candida auris* is an emerging pathogen of great clinical concern. 90% of clinical *C. auris* isolates are resistant to fluconazole (FLU), yet it remains unknown what mechanisms underpin FLU resistance in *C. auris.*

Methods: FLU evolved strains were generated by passaging *C. auris* clinical isolate AR0387 (FLU minimum inhibitory concentration [MIC] 1mg/L) in YPD supplemented with FLU. FLU MIC were measured by broth microdilution. Sanger sequencing of the *C. auris* homologs of *Candida albicans* transcriptional regulator genes and ergosterol biosynthesis genes was performed. The relative expression of homologs of *C. albicans* FLU resistance effectors was assessed for each strain by RTqPCR. FLU resistance associated mutations of interest were tested using a Cas9-mediated transformation system.

Results: FLU MIC for the 5 evolved strains ranged from 8 to 64mg/L. All evolved strains were found to harbor mutations in *TAC1B*. RTqPCR revealed all evolved strains to exhibit a 3 to 5-fold increase in the relative expression of *CDR1*. Subsequently, mutations in *TAC1B* were also identified among >60% of 231 fluconazole-resistant *C. auris* clinical isolates from a global collection. Introduction of the most common *TAC1B* mutation found among clinical isolates into AR0387 resulted in a 16-fold increase in FLU MIC, and correction of this same *TAC1B* mutation to the wildtype sequence in a fluconazole-resistant clinical isolate resulted in a 16-fold decrease in FLU MIC.

Conclusions: These studies demonstrate that *C. auris* can rapidly acquire resistance to FLU *in vitro*, and that mutations in *TAC1B* contribute to clinical FLU resistance. Further research is needed to characterize the *C. auris TAC1B* regulon.

075B

EVALUATION OF ANTIFUNGAL ACTIVITY OF TERMINALIA SUBSPATHULATA BARK AND FRUIT EXTRACTS AGAINST AZOLE-RESISTANT CANDIDA ISOLATES

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Abstract

Background: Emerging resistant *Candida* species become a severe problem for public health. The study aims to examine the efficacy of *Terminalia subspathulata* bark and fruit extracts as an alternative source of antifungals for resistant strains of *Candida* species.

Methodology: *Candida albicans* was isolated between 4–5th interdigital foot space and *C. parapsilosis* were isolated from the left-hand third finger of male patients. Morphological and molecular methods identified the isolates. Antifungal susceptibility testing (AFST) was determined using azole derivatives, amphotericin B (AmpB) and echinocandins based on E-test and broth microdilution (BMD) methods. The AFST activity of the 10 µL methanolic extracts with concentrations between 1.25–10 mg/mL was assessed by disk diffusion method according to CLSI guidelines.

Results: The AFST results showed total resistance to all azoles (FLU, MIC ≥ 256 µg/ml and VOR, ITR and POS each with MIC ≥ 32 µg/ml). The BMD method agreed with E-test MICs. The *T. subspathulata* bark extract tested indicated activity on the *C. albicans* lawn with the zone of inhibition between 9–13 mm. The fruit extract indicated zone between 8–10 mm. *Candida parapsilosis* indicated susceptibility to bark extract with the zone of inhibition between 10–15 mm, while the fruit extract showed zone between 8–13 mm.

Conclusion: Despite azole resistance, the extracts showed good activity against the isolates. Findings showed alternatives to AmpB and echinocandins because of toxicity and parenteral administration, respectively. Based on our findings, our study first reports the medicinal value of *T. subspathulata*.

076C

High Throughput Screening of the Calibr ReFRAME and the Broad Institute Repurposing Hub Libraries to Identify Inhibitors of *Candida albicans* Biofilm Formation

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Abstract

Candida albicans is an opportunistic pathogenic fungus affecting immune or medically compromised individuals. Candidiasis is the third to fourth most common hospital-acquired infection in the United States. *C. albicans* infections are often associated with biofilm formation, which contributes to its ability to resist antifungal treatment, increasing the urgency to find new anti-biofilm therapies. In order to identify compounds with the ability to inhibit biofilm formation, we developed a new technique for *C. albicans* biofilm formation in wells of 384 microtiter plates and used it to conduct high throughput screenings (HTS) of two drug repurposing libraries: the ReFRAME collection (>12,000 compounds) provided by California Institute of Biomedical Research, and the Drug Repurposing Hub (>6,000 unique compounds) provided by the Broad Institute. Setting a threshold of >70% inhibition, the initial HTS of the ReFRAME library identified 104 hits, of which 101 compounds were confirmed in secondary dose-response assays, including 21 compounds not classified as antiseptics, antibacterials or antifungals. The HTS of the Drug Repurposing Hub resulted in the identification of 57 hits, with dose-response assays confirming the anti-biofilm activity of 56 compounds, 24 of which are not classified as antiseptics, antibacterials or antifungals. To our knowledge, this constitutes the largest screening of repurposing libraries to date in search for repositionable compounds in the antifungal space. After subsequent experimentation, the leading compounds could potentially be repurposed as antifungals to combat *C. albicans* biofilm infections, and potentially other fungal infections.

077A

Novel antifungal activity of Q-Griffithsin, a broad-spectrum antiviral lectin

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Abstract

Background

There is a rising global trend in candida strains with high resistance to fluconazole and other antifungal drugs, hence the need for novel agents. Here, we investigated the anti-Candida activity of Q-Griffithsin (Q-GRFT), a lectin naturally produced by the red-sea algae, *Griffithsia spp*.

Methods

To assess *in vitro* growth inhibitory activity, *C. albicans* was incubated with Q-GRFT on agar plates and in broth media. We investigated GFP-bound Q-GRFT's ability to adhere to *C. albicans* using fluorescence microscopy and fluorescence intensity assessments. To demonstrate *in vivo* growth inhibitory activity, CBA/J mice were treated *per vaginam* with Q-GRFT followed by challenge with *C. albicans,* and fungal burden determined following vaginal lavage.

Results

Wild type fluorescently labeled Q-GRFT displayed higher fluorescence than the lectin-binding site deficient variant following incubation with *C. albicans.* Q-GRFT localized around the fungal cells and bound to α-mannan in the cell wall. Q-GRFT significantly inhibited *C. albicans* growth in broth and on agar plates, disrupted the integrity of the cell wall, and induced ROS formation. The lectin significantly inhibited the growth of *C. glabrata*, *C. parapsilosis* and *C. krusei*, with modest activity against *C. auris* CDC388 and *C. auris* CDC389 strains *in vitro*. Topical treatment resulted in a lower fungal burden compared to the vehicle control group in vaginal candidiasis.

Conclusion

Q-GRFT binds to and inhibits *C. albicans* growth both *in vitro* and *in vivo*. Further studies are needed to establish the mechanism of growth inhibition.

078B

Molecular mechanisms of pan-azole resistance in *Candida haemulonii* species complex from Brazil

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Abstract

The *Candida haemulonii* complex and related emergent species are composed by emergent fungal pathogens able to cause invasive infections with high rates of clinical treatment failures. In addition, outbreaks in hospital settings have been reported worldwide, leading to high mortality associated with these infections. The *C. hameulonii* clade isolates typically demonstrate resistance to the first-line antifungal agents as fluconazole. Twelve non-susceptible *Candida haemulonii* complex clinical isolates were investigated. CLSI antifungal susceptibility testing of five azoles were performed. Efflux pump activity were assessed biochemically by Rhodamine 6G. Efflux pump inhibitor (EPI) was used to assess the contribution of these transporters to fluconazole sensitivity. The *ERG11* gene was amplified and sequenced to identify amino acid polymorphisms, while real-time PCR was utilized to investigate the expression levels of *ERG11, CDR1, CDR2* and *MDR1* orthologous. Azole cross-resistance was detected in all except one isolate. *C. haemulonii* exhibited higher ATP-dependent drug efflux activity than other non-*albicans Candida* species. When this activity was inhibited, fluconazole sensitivity was enhanced 8-to more than 64-fold. Twelve amino acid substitutions were observed *ERG11* gene. Overall, this study provided significant evidences of the importance of drug efflux pumps activity in mediating azole resistance in *C. haemulonii*.

079C

Genome-scale metabolic models for *Candida* species: a platform for drug target prediction

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Abstract

Candida species are among the most impactful fungal pathogens, normally associated with very high mortality rates. With the rise in frequency of multidrug-resistant clinical isolates, the identification of new drug targets and new drugs is crucial to overcome the increase in therapeutic failure. Here, we present the first validated genome-scale metabolic model for *C. albicans*, iRV781. The model was reconstructed using the open-source software tool *merlin* 4.0.2, and validated, proving accurate when predicting the capability of utilizing different carbon and nitrogen sources when compared to experimental data. The genome-scale metabolic reconstruction was tested as a platform for the identification of drug targets, through the comparison between known drug targets and the prediction of gene essentiality in conditions mimicking the human host. The extension of this approach to additional *Candida* species is underway. Altogether, these models provide a promising platform for global elucidation of the metabolic potential of *Candida* species, with expected impact in guiding the identification of new drug targets to tackle human candidiasis.

080A

Identification and characterization of a fungal-selective glutaminyl tRNA synthetase inhibitor with potent activity against *Candida albicans*

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Abstract

Candida albicans is the leading cause of systemic candidiasis. Effective treatment is threatened by a dearth of antifungal options and the emergence of resistance. Thus, there is an urgent need to identify novel therapeutic targets to expand our antifungal armamentarium. A promising approach is the discovery of essential genes, as most antimicrobials target essential bioprocesses. Despite detailed characterization of gene essentiality in *Saccharomyces cerevisiae,* defining essential targets in the pathogen of interest is necessary due to the high level of divergence between these organisms. Thus, using a machine learning algorithm we generated a comprehensive prediction of all genes essential in *C. albicans.* We leveraged our essentiality predictions with high-throughput screening and chemogenomic datasets to assign the mechanism of action of a previously uncharacterized compound. We identified T-035897 as a molecule with potent bioactivity against *C. albicans*. Prior chemogenomic profiling in *S. cerevisiae* suggested that T-035897 targets the glutaminyl tRNA synthetase Gln4, whose homolog in *C. albicans* was predicted and verified to be required for viability. To confirm the mechanism of T-035897 in *C. albicans*, we performed haploinsufficiency profiling, which supported Gln4 as the target. In parallel, selection of resistant mutants and targeted sequencing uncovered substitutions in the Gln4 catalytic domain. Moreover, T-035897 inhibited translation in a fluorescence-based reporter assay. Finally, T-035897 selectively abrogated fungal cell growth in a co-culture model with mammalian cells. Thus, we highlight the power of leveraging essentiality datasets in order to characterize compounds with potent antifungal activity in an effort to unveil novel therapeutic strategies.

081B

Identification of a novel lipid biosynthesis inhibitor with activity against the emerging fungal pathogen *Candida auris*

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Abstract

Candida species are amongst the most prevalent causes of systemic fungal infections, which account for over one million annual fatalities and pose a growing threat to public health. *Candida albicans* represents the most common etiological agent; however, the rate of infections caused by non-*albicans Candida* species continues to rise. Among these emerging drug-resistant pathogens is *Candida auris*, which since its discovery in 2009, has been identified worldwide and exhibits resistance to all three antifungal classes: the azoles, polyenes and echinocandins. Here, we endeavoured to identify compounds with novel bioactivity against *C. auris*. Leveraging compound libraries through screening for antifungal activity against *C. auris*, we prioritized a novel single agent bioactive from the Medicines for Malaria Venture's Pathogen Box library, MMV688766. Chemogenomic profiling unveiled that heterozygous deletion of the fatty acid synthetase gene, *FAS1,* as well as sphingolipid biosynthesis genes, *AUR1* and *LCB2,* resulted in hypersensitivity to MMV688766, suggesting this compound modulates fungal lipid homeostasis. In support of this model, transcriptional repression of additional genes involved in sphingolipid and sterol biosynthesis resulted in hypersensitivity to MMV688766. Finally, untargeted lipidomics profiling revealed that cells treated with MMV688766 demonstrate dramatically reduced levels of sphingolipid intermediates, including ceramides and inositol-phosphorylceramides. Increased endpoint levels of mannosyl-diinositol-phosphorylceramides, as well as both lanosterol and ergosterol were also observed in the treated population. Future work will focus on defining the precise target of MMV688766 using a variety of biochemical approaches. Collectively, this work highlights a molecule with efficacy against an emerging fungal pathogen, which may pave the way for future antifungal drug development.

082C

Eradication of *Candida albicans* biofilm by dendritic molecules

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Abstract

Candida spp. are fungal opportunistic pathogens of clinical significance with the ability to form biofilms. Medical devices, such as catheters or prostheses, are common niches where biofilms manage to develop and provide resistance to antifungal compounds. Thus, *Candida* biofilms are an important reservoir of infections. Besides, resistance and lack of effective molecules against biofilms make necessary to develop new biocides to prevent and treat *Candida* biofilm-related infections. In this regard, dendritic compounds have emerged as an exciting new class of microbicidal agents with low cytotoxicity. *In vitro* activity of three molecules (BDTL53, BDTL56 and BDTL59) was tested against biofilm formation and stablished biofilms of a CECT *C. albicans* strain. Dendrimers with the highest antifungal activity were also studied in combination with silver nitrate (AgNO3) and ethylenediaminetetraacetic acid (EDTA). Cell viability was evaluated using resazurin colorimetric assay and confirmed by plating on agar. Cytotoxity was studied in human cell lines, and biofilm alterations were observed by scanning electron microscopy (SEM). BDTL59 was the most active compound preventing biofilm formation, showing a minimum biofilm inhibitory concentration (MBIC) of 16 mg/L. The minimum biofilm damage concentration (MBDC) against stablished biofilms was 128 mg/L. However, growth was observed on agar plates, which showed that biofilm cell population was not completely eradicated at this concentration. SEM micrographs showed reduction on biofilm density and alterations on cell morphology. Synergy was also detected and these combinations prevented biofilm formation and eradicated stablished biofilms. We conclude that dendritic molecules may be a new alternative to treat *C. albicans*.

083A

Identifying and Characterizing a Fungal Peptide that Attenuates *Enterococcus faecalis*

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Abstract

Candida albicans and *Enterococcus faecalis* are opportunistic pathogens that co-exist in the human gut, urogenital tract, and oral cavity. The fungus and bacterium are common causes of life-threatening infections that afflict immunocompromised individuals and are frequently isolated together. The relationship between *C. albicans* and *E. faecalis* is complex, antagonistic, and not fully characterized. This relationship has the potential to be exploited and used as a basis for anti-bacterial therapies against multi-drug resistant *Enterococci*, which are increasingly common. Using a *Caenorhabditis elegans* infection model, it was previously demonstrated that co-infection with *C. albicans* attenuates *E. faecalis* virulence. We have shown that supernatants isolated from dual-species biofilms of *C. albicans* and *E. faecalis* reduce the ability of *E. faecalis* to form biofilms and kill *C. elegans*. Supernatants from single species *C. albicans* or *E. faecalis* cultures or biofilms lack this activity. Based on our preliminary characterization, we hypothesize that *C. albicans*, upon exposure to *E. faecalis*, produces a small peptide that hinders the virulence of the bacterium. To identify the active compound, we screened a *C. albicans* mutant library for strains that failed to generate this activity when grown in the dual-species biofilms. Detailed characterization of several *C. albicans* mutants that are unable to repress *E. faecalis* biofilm formation is ongoing to identify the peptide that governs this inhibition, after which the mechanism of action will be determined. This unidentified fungal peptide has the potential to become a novel or adjunctive therapy in the treatment of antibiotic resistant *E. faecalis* infections.

084B

A Comparative Study of Boric Acid and Fluconazole Drug Response in C. albicans Planktonic and Biofilm Cells

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Abstract

Candida albicans is the most prevalent fungal pathogen that causes vulvovaginal candidiasis (VVC) and recurrent VVC (RVVC, defined as four or more episodes in a year) though incidence of non-albicans (NAC) species are increasing. Although the azole drug fluconazole (FLC) is the primary antifungal drug used to treat VVC/RVVC, NAC species isolates often have intrinsic resistance to FLC. Moreover, Candida biofilms have previously been shown to be insensitive to FLC, and biofilms may be involved in VVC/RVVC infections. The broad-spectrum antimicrobial boric acid (BA) is also used to treat VVC; however, it is considered as a second-line treatment and much less is known about how it inhibits Candida spp. We compared the efficacy of FLC and BA against planktonic cells, in reducing biofilm formation, and against preformed biofilms in diverse clinical strains and multiple Candida species. We found that NAC species showed lower susceptibility to both FLC and BA and higher intrinsic tolerance to BA than C. albicans; however, the magnitude of difference in BA was much lower than FLC. In all species, the population-level variation for resistance and tolerance was broader for FLC than BA. Unlike FLC, BA inhibited C.albicans biofilm formation in a dose-dependent manner. BA effectively reduced the biomass and metabolic activity of preformed biofilms, while FLC at all concentrations did not inhibit biofilm growth. Our findings suggest that BA is effective against Candida spp. planktonic cells, inhibits biofilms formation and eradicates preformed biofilms, which could explain why it is effective against VVC/RVVC.

085C

Proteomic study reveals the importance of Calmodulin-calcineurin pathway after caspofungin exposure in resistant C. glabrata: CaM / Cal inhibitors as a repurposing strategy

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Abstract

Echinocandin resistance is a great concern, considering that these drugs are recommended as first-line therapy for invasive candidiasis. Its resistance is conferred by mutations in *FKS* genes. Nevertheless, some pathways which regulate cellular stress responses could be crucial for enabling tolerance, evolution, and maintenance of drug resistance. To improve clinical outcomes, development of new antifungal drugs with different mechanisms of action, and/or to discovery of drugs which improve the activity of current drugs are urgently needed.

In this work, the proteomic response to caspofungin exposure in echinocandine-resistant *C. glabrata* was investigated. Results showed that some up-regulated proteins are involved in cell wall biosynthesis, response to stress and pathogenesis, some of them being members of calmodulin-calcineurin pathway. Therefore, the impact of calmodulin and calcineurin inhibitors (Fluphenazine, tacrolimus, cyclosporin) on susceptibility, stress tolerance, biofilm formation, and pathogenicity in caspofungin-resistant *C. glabrata* clinical isolates was explored. These inhibitors allow caspofungine susceptibility restoration, decrease of capacity to respond to stress conditions, reduction of biofilm formation and *in vivo* pathogenicity. In conclusion, in a repurposing strategy, our findings confirm that calmodulin-calcineurin pathway could provide a relevant target for life-threatening fungal diseases and that new and more selective compounds without immunosuppressive adverse effects could be developed.

086A

in vivo virulence and genomic insights of azole-resistant clinical *Candida albicans* from the Philippines

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Abstract

Information in regard to the status of *C. albicans* in the Philippines is limited due to the lack of active surveillance, fungal culturing and identification from clinical specimens.Increase in antifungal resistance is to be anticipated due to the rising cases of human immunodeficiency virus (HIV), and candidiasis as one of the most common fungal infections in the country. Twenty-six *C. albicans* strains were isolated from clinical specimens (i.e. throat swab, sputum, skin, and colonoscopy drain), in which 19 (73.08%) were fluconazole-resistant. This research sequenced the genomes of seven *C. albicans* and mapped the genomes against the reference strain SC5314. Of the seven isolates, five were tested to be resistant based on *in vitro* MIC assays with MIC value of at least ≥8ug/mL and two isolates were susceptible (≤0.125ug/mL).Variant-calling analyses revealed non-synonymous mutations in genes associated with the ergosterol pathway (i.e. *ERG3* and *ERG11*) and novel mutations in the transcriptional factors *MRR1*, *UPC2,* and *TAC1* that were only identified in resistant strains. Using the *Galleria mellonella* model, infection studies resulted to clinical isolates being more pathogenic than SC5314, and that *in vitro* azole resistance among the clinical isolates does not necessarily correlate with resistance *in vivo.* Studies on the efficacy of fluconazole at a concentration of 3.2 µg fluconazole/larva revealed a protective effect against infection by majority of the isolates which were identified to be resistant to the drug *in vitro.* This study shows genomic variations in clinical *C. albicans* from clinical samples in the Philippines, likewise the use of *Galleria* as host in studying virulence and understanding how these isolates interact with the host.

087B

Precise editing using CRISPR-Cas9 to explore the contribution of clinically-derived mutations to antifungal resistance in the pathogenic yeast *Candida parapsilosis*

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Abstract

Introduction

Candida parapsilosis is both a commensal/saprophytic yeast of the human skin and an opportunistic pathogen which can be responsible for life-threatening infections. The increasing reports of clonal outbreaks involving azole-resistant *C. parapsilosis* in the clinical setting is worrisome and urges for a better understanding of antifungal resistance in this species. Previous studies have identified mutations in key genes which can explain acquired fluconazole resistance. Reverse genetics approaches are now warranted to confirm their involvement and to determine whether they can affect other clinically-licensed antifungals. Here, we used a CRISPR-Cas9 technique to study the relative contributions of clinically-derived mutations to antifungal resistance and provide answers to these questions.

Materials and Methods

Six clinically-derived mutations were selected ($ERG11^{Y132F}$, $ERG11^{K143R}$, $ERG11^{R398I}$, $TAC1^{G650E}$, $MRR1^{G583R}$, $ERG3^{G111R}$) to be engineered in two *C. parapsilosis* fluconazole-susceptible backgrounds (ATCC22019, STZ5) using a previously described CRISPR-Cas9 method. *In vitro* susceptibility of the transformants to fluconazole, voriconazole, posaconazole, isavuconazole and micafungin was determined by Etest®.

Results/Discussion

The impact on fluconazole susceptibility was highly variable depending on the residue/gene involved, but roughly similar between the two genetic backgrounds. All but two ($ERG11^{R398I}$, $ERG3^{G111R}$) conferred fluconazole resistance, though the highest MIC increase was observed for $MRR1^{G583R}$ (≥650 fold). As expected in a diploid species, we noted an impact of allelic dosage. Some kind of cross-resistance to the other azoles was noted from some mutations, although the impact was lower for posaconazole and isavuconazole, except for $MRR1^{G583R}$ which led to multi-azole resistance. Finally, $ERG3^{G111R}$ increased tolerance to both azoles and echinocandins.

088C

Inhibition of Hsp90–Cdc37 interaction support the Candida antifungal activity of the natural compound Celastrol

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Abstract

During the past decade systemic candidiasis incidence has increased placing the yeast *Candida albicans* at the fourth rank of pathogenic agent responsible for hospital-acquired infections. Host toxicity, drug interactions, paucity of target and emergence of resistance limit the current treatment strategies. To overcome these limitations as well as to improve our therapeutic armamentarium, original targets need to be identified to develop new antifungal alternatives. In *C. albicans* the Heat Shock Protein 90 is a major regulator of virulence and resistance. Previous studies identified Celastrol as a compound disrupting the human Hsp90-Cdc37 interaction involved in PKC signaling pathway.

Celastrol has been tested *in vitro* against one ATCC strain (SC5314) and 20 clinical isolates representing the five more common *Candida* species (C. *albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei*) by a spectrofluorometric microdilution method and the IC_{50} were calculated. The mechanism of action of Celastrol has been investigated with western blotting analysis of Hsp90, Cdc37 and PKC expressions in *C. albicans* after treatment.

Celastrol has shown anti proliferative activity against all the five common species of *Candida* sp*. in vitro* with IC_{50} range of 1.8-27 µM with higher activity against less susceptible or resistant-fluconazole *C. glabrata* and *C. krusei* respectively*.* Western blotting validated that Celastrol disrupts the Hsp90-Cdc37 complex, leading to decrease in the expression and/or to degradation of the client-protein PKC.

Antifungal activity of Celastrol involves the disruption of Hsp90-Cdc37 complex. Targeting of the yeast co-chaperone Cdc37 is an interesting approach for development of innovative and more selective antifungal drugs.

089A

Zinc binding by Histatin 5 promotes cell surface damage in *C. albicans*

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Abstract

Background. Histatin 5 (Hst5) is a metal-binding human salivary protein with potent fungicidal activity. Under metal-free conditions, Hst5 disrupts *Candida albicans* intracellular functions but is not membrane-lytic. However, its activity within the zinc-rich oral environment is unknown.

Objectives. Zinc induces Hst 5 to dimerize and improves fungal membrane binding, so we hypothesized that Zn-Hst5 complexes potentiate fungal membrane disruption.

Results. We compared Hst5 with a low zinc-affinity variant, Hst5ΔMB (Δ1-3->Glu and Δ15-19->Glu) and found that Hst5-Zn complexes had increased fungicidal activity compared to Hst5 alone. Hst5ΔMB had low activity with or without Zn. To assess membrane disruption, ATP efflux from *C. albicans* cells was measured over 1-10min of Hst5 exposure using a Staybrite ATP kit (Biovision). Hst5-Zn induced 3.7-fold more ATP efflux within 1min than Hst 5, while Hst5ΔMB caused minimal ATP efflux, suggesting Hst5-Zn promotes membrane disruption. *C. albicans* responds to membrane stress by three major pathways (HOG, PKC, and CEK) whose activation was examined following Hst5-Zn exposure. Hst5-Zn induced sustained phosphorylation of Mkc1 (PKC pathway) compared to Hst5, but Cek1 and Hog1 induction was indistinguishable. *C. albicans MKC1* deletion mutants were more susceptible to killing by Hst5-Zn but not to Hst5 alone. As Mkc1-P regulates chitin production, we measured cell-wall chitin using calcofluor white staining and flow cytometry and found chitin was increased 25% in Hst5-Zn treated cells compared to Hst5 alone, suggesting a cell-wall stress response.

Conclusions. Hst5-Zn induces cell-surface damage and ATP release, either directly with the cell membrane or by weakening the cell wall.

090B

In vitro evolution of multidrug and cross resistance in different clades of *Candida glabrata*

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Abstract

Currently, there is only a few drug families available to treat fungal infections, and the number of strains resistant to one or several drugs is increasing. This issue is particularly problematic in *Candida glabrata*, which is intrinsically less susceptible to azoles and often acquires resistance to azoles and/or echinocandins. Although genetic resistance in this species has been attributed to mutations in drug target genes, *FKS* genes for echinocandin resistance, and a transcription factor, *PDR1* controlling the expression of drug transporters in azole resistance, we sought to unravel alternative resistance-conferring mutations and mutational paths that could lead to the acquisition of resistance. We performed an in vitro evolution experiment by exposing genetically distinct strains of *C. glabrata* to increasing concentrations of an azole and an echinocandin and combinations thereof. Here, we present results on drug susceptibilities and fitness. Our results show that simultaneous exposure to the two drugs may be advantageous in both the efficiency of the treatment and in reduction of the possibility of the acquisition of drug resistance. All surviving strains acquired resistance to the exposed agent(s) and in serial drug exposure experiments, previously acquired resistance was rarely lost during exposure to the second drug. Interestingly, we observe increased resistance to fluconazole in a large subset of samples evolved with anidulafungin indicating that adaptation to anidulafungin can induce cross resistance to fluconazole. Further, whole genome sequencing of selected strains will help us define the mutational paths that lead to the acquisition of resistance.

091A

ID-CARD: A clade-specific molecular assay for the detection of 25 Candida spp. causing candidemia based on antifungal susceptibility patterns and in vitro testing of the antifungal activity of a synthetic antimicrobial peptide derived from human lactoferrin (hLF1-11).

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Abstract

Fungal infections are a serious health concern affecting over 1.5 million individuals annually. ID-CARD aims to improve diagnostics taking into account phylogeny and antifungal susceptibility patterns of *Candida* spp. involved in candidemia.

Twenty-five *Candida* spp. were chosen. Based on ribosomal DNA sequences, clade-specific primers/Taqman probes were designed. Different multiplex panels consisting of four clades that exhibited similar antifungal susceptibility profiles were created. To create the groups, we tested fluconazole and anidulafungin with broth microdilution according to EUCAST against 3-5 isolates/species (n=121), which were also used for specificity testing of the molecular assay. Furthermore, we tested the *in vitro* activity of hLF(1-11) peptide against isolates that exhibited elevated minimum inhibitory concentrations (MICs) for one or both of the drugs.

The groups created are : i. Lodderomyces, Kluyveromyces, Metschnikowiaceae Sensitive, Internal control, (all with low MICs) ii. Pichiaceae, Nakaseomyces, Wickerhamomycetaceae, Debaryomyces & Diutina, (all with high MICs to azoles) and iii. Yarrowia, Wickerhamiella & Meyerozyma, *Candida auris*, *Candida haemulonii* complex (all with high MICs to both azoles & echinocandins). The primers/probes showed 100% specificity and capacity for multiplexing. *In vitro* experiments indicated that hLF(1-11) is fungicidal against various *Candida* spp. A synergistic effect of antifungal and hLF(1-11) against various *Candida* species was shown as combinations of the peptide with antifungals were more effective than these alone

ID-CARD will contribute to a fast and reliable molecular detection of yeasts involved in candidiasis. AMPs is a novel way to treat *Candida* spp. exhibiting high MICs to commonly used antifungal drugs.

092B

Candida albicans and *Candida dubliniensis* behave differently when exposed to Echinocandins and Azoles

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Abstract

Background

Candida albicans and *Candida dubliniensis* are closely related species and exhibit the same antifungal susceptibility patterns. However, we anecdotally observed differential growth patterns between these two species when they were tested by the Etest method for susceptibility to fluconazole and echinocandins. Here, we further explored this observation.

Materials and Methods

Sixty clinical isolates of the two species were tested against fluconazole and echinocandins by the reference EUCAST technique and by the Etest method, in order to evaluate differences between their growing patterns. In particular we analyzed trailing *ie* incomplete growth inhibition at supra-MICs.

Results

For *C. albicans*, trailing was observed with fluconazole in 90% and 93.3% for EUCAST and Etest, respectively, but not with echinocandins (<7% for EUCAST and 0% for Etest). In contrast, for *C. dubliniensis*, trailing was very rarely observed with fluconazole (20% for EUCAST and 0% for Etest), while the opposite pattern was observed with echinocandins (>50% for EUCAST and > 86% for Etest).

Conclusion

C. albicans and *C. dubliniensis* isolates exhibit independently of the methods used for susceptibility testing differential patterns of growth against fluconazole and echinocandins. This could suggest that the pathways involved in trailing differ between the two species, despite their phylogenetic proximity. Clinical microbiologists must be aware of these species-specific patterns for reliable MIC determinations.

093C

Targeting the Candida albicans' "Achilles heel" in search for novel drug target

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Abstract

The fungal cell wall is essential for cell viability and is composed largely of molecules that are absent in mammals. As a result, cell wall biosynthetic enzymes have great potential specific antifungal drug targets. Importantly, these enzymes that are involved in the assembly of the major polysaccharides that represent the major structural elements of the wall require a steady supply of cytoplasmic sugar nucleotide donors. Here we take a multidisciplinary approach to genetically and chemically validate *C. albicans* enzymes involved in the synthesis of these sugar nucleotide precursors as antifungal targets. Using CRISPR-Cas9 technology we generated conditional mutants that allowed regulation of potentially essential genes encoding sugar nucleotide biosynthetic enzymes. Of the mutants tested, we identified two genes – *AGM1* and *GFA1* as being essential for growth. These enzymes possess human orthologues therefore the conditional mutants of *C. albicans* were complemented by knocking in the human orthologuous gene, thus creating a set of "humanised" *C. albicans* constructs to test on-target selectivity and off-target toxicity of new antifungal compounds. For chemical validation a fragment-based design approach was used to synthesize unique compounds that specifically bind fungal enzymes that were distinct from substrate analogues. These tools will serve as invaluable tools to identify novel antifungal drug structures against essential processes in fungal cell wall assembly.

095B

Proteomic study of the effect of metformin on *C. albicans*

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Abstract

Fungal infections are a global health problem. Of them, those produced by *Candida albicans* are the most important, with a reduced arsenal of antifungals and an increasing problem of antifungal resistance. Thus, the discovery of new antifungal targets and drugs remains interesting. Metformin is a biguanide administered as a first-line treatment for Type II Diabetes Mellitus and it has recently been published its anti-*Candida* action, especially against *C. glabrata*, and its synergistic effect with other antifungals. Our studies of the effect of metformin on *C. albicans* have revealed an inhibition of growth, filamentation and other phenotypes important for virulence. Although metformin has been described as an AMPK agonist, its mechanism of action is partly unknown. To deepen into the anti-*Candida* mechanism of action, we have addressed the differential proteomic study. A set-up of the conditions for the proteomic study has been carried out, fixing a concentration of 50mM of metformin, 6 h of treatment at 37ºC in RPMI medium and with 60 rpm of agitation. The proteomic study using the *Labelfree* technique and 4 biological replicas, allowed the identification and quantification of a total of 1899 proteins, 206 of them presenting differences in abundance due to metformin exposure. Of these, 127 increased and 79 decreased due to the action of the drug. The most relevant functions of these proteins are related to antifungal response, filamentation, biofilm formation and metabolism, being 9 essential proteins for the microorganism that could be new antifungal targets.

096C

Candida glabrata MSH2 deletion increases antifungal tolerance in vitro and during intra-abdominal candidiasis (IAC), it does not impact pathogenesis of peritonitis or intra-abdominal abscesses.

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Abstract

Background: IAC is the second most common type of invasive candidiasis, but its pathogenesis is poorly understood. We have shown that *Candida albicans* DNA damage response genes are strongly induced within intra-abdominal abscesses. Deletion of *C. glabrata* MSH2, a DNA mismatch repair (MMR) gene, results in a mutator phenotype that facilitates multidrug resistance *in vitro* and in mouse gastrointestinal tracts. Our goal was to determine if CgMSH2 contributed to pathogenesis or resistance to the new antifungal rezafungin during IAC.Methods: We created Δ*msh2* in BG2 using SAT-flipper, and tested virulence and rezafungin responses in a mouse model of IAC.Results: Δ*msh2* displayed no growth defects at 30○C in liquid (YPD, YPGlycerol) or solid media (YPD+0.02% MMS, 1mM H2O2, 1M NaCl, 20 ug/ml CW, 250 ug/ml or 0.02% SDS). *Δmsh2* longevity in YPD was comparable to BG2. Caspofungin-, rezafungin- and fluconazole-resistant mutants arose 24-, 16- and 3-fold more often, respectively, for Δ*msh2* than BG2 (10^8-10^6 CFU overnight in YPD, selected on 8xMIC-containing plates). However, respective minimum inhibitory concentrations (MICs) were not different, nor were rezafungin time-kills. *Δmsh2* was comparable to BG2 in peritonitis and abscess burdens in mouse IAC. *Δmsh2* demonstrated significantly greater caspofungin- and fluconazole-tolerance than BG2 in abscesses. Rezafungin reduced peritonitis and abscess burdens of *Δmsh2,* BG2 and *fks* mutant strains to similar extents.

Conclusions: CgMSH2 deletion increased the frequency of spontaneously-arising echinocandin- and fluconazole-resistant colonies *in vitro* and tolerance in intra-abdominal abscesses, but it did not attenuate virulence or rezafungin responses during IAC.

098B

Comprehensive qPCR diagnostic assays for the *Candida auris* surveillance

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Abstract

Background

Rapid detection and accurate identification of *Candida auris* together with an assessment of antifungal drug resistance are essential for effective patient management, and infection prevention and control in healthcare facilities. Here, we developed and validated platforms for direct detection of *C. auris* and its *ERG11*-associated azole resistance and *FKS1*-associated echinocandin resistance in clinical skin swabs.

Methods

DNA isolated from 112 de-identified clinical axilla/groin skin swabs was tested in *C. auris*-specific SYBR Green qPCR assay. *C. auris*-positive and *C. auris*-spiked samples were further analyzed in the resistance markers assay. Enrichment culture followed by rDNA sequencing, antifungal susceptibility testing (AFST; CLSI guidelines) with echinocandins and azoles, and sequencing of *ERG11*, and *FKS1* genes were used as reference methods.

Results

Detection of *C. auris* in qPCR assay was not affected by other microorganisms present in the sample. The assay produced results comparable to the enrichment culture approach currently implemented at the CDC. The results of the resistance markers assays were 100% concordant with gene sequencing and AFST results. Genotyping confidence for *FKS1* and *ERG11* was obtained at the level of 10^5 CFU/ml and 10^4 CFU/ml, respectively.

Conclusion

We found that the *C. auris*-specific qPCR assay can be successfully applied for rapid and accurate detection of *C. auris* in skin swabs, thereby increasing high-throughput diagnostic options for *C. auris* surveillance. The resistance markers assay performed well in categorizing swabs as harboring wild-type or mutant *C. auris,* and thus, it holds promise as a surrogate diagnostic method to direct antifungal therapy more effectively.

100A

Combining colistin and fluconazole to increase fungal membrane permeability and antifungal cidality in highly tolerant Candida albicans

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Abstract

The current clinical arsenal for invasive *Candida* diseases is inadequate to treat the spectrum of clinically insensitive isolates that are constantly emerging. In particular, there is significant resistance and tolerance associated with the fungistatic drug fluconazole in a number of *Candida* species. A promising option to expediate the development of novel antifungal treatment regimens is to repurpose existing drugs. Here we show that combining fluconazole with clinically relevant doses of the antibiotic colistin induced fungicidal activity in a range of fluconazole-susceptible and -resistant isolates. Colistin reduced the minimum inhibitory concentration of fluconazole by four-fold *in vitro*, and was associated with increased survival in a *C. albicans Galleria mellonella* pathosystem. This combination was particularly effective in highly fluconazole tolerant *C. albicans* species, which is potentially highly relevant as this subpopulation of cells may be associated with persistent infections. Colistin is a positively charged lipopeptide that directly binds to several membrane lipids (e.g. L-α-phosphatidylinositol, L-α-phosphatidyl-L-serine and L-α-phosphatidylethanolamine) that are enriched in ergosterol-depleted cells, the target pathway of fluconazole. Colistin increases the permeability to fluorescent azole probes and induced an increase in cell death that was dependent on ergosterol depletion. This indicates that colistin permeabilizes cells by binding to membranes that are depleted of ergosterol, whether by azole antifungal treatment or ergosterol biosynthesis mutations. Overall, colistin permeabilises fungal membranes through binding to lipids in membranes depleted of ergosterol.

101B

Novel chromogenic media for the detection and identification of multi-drug resistant *Candida auris*

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Abstract

An increasing amount of drug resistant fungal species has been isolated in the nosocomial environment. The multidrug-resistant species *Candida auris* is one of the most notorious examples. Within a decade *C. auris* has spread globally and causes life-threatening infections. Fast and accurate species identification has become essential. Chromogenic media are easy-to-use and allow for rapid *Candida* identification based on colony phenotypes. We evaluated two novel chromogenic formulations, HiCrome™ *C. auris* MDR Selective Agar (HiMedia) and CHROMagar™ *Candida* Plus (Chromagar), and three reference media, CandiSelect™ (Bio-Rad), Chromatic™ *Candida* (Liofilchem) and CHROMagar™ *Candida* (Chromagar), for their performance to isolate and detect *C. auris* and its close relatives. Media were inoculated with a collection of genetically diverse *C. auris* strains (n=9) and closely related comparator species (n=35). After 48h of incubation the media were assessed for their ability to detect and identify *C. auris*.

All media had similar limitations with respect to differentiation of *Candida glabrata* and *Candida dubliniensis.* A species-specific coloration for *C. auris* was only observed on CHROMagar™ *Candida* Plus, while the general media and HiCrome™ *C. auris* MDR Selective Agar were unable to do so. However, CHROMagar™ *Candida* Plus was not able to differentiate *C. auris* from its pathogenic siblings *Candida pseudohaemulonii* and *Candida vulturna* as they had all three a similar appearance.

Overall, CHROMagar™ *Candida* Plus showed a better performance in the detection and identification of *C. auris*, but *C. pseudohaemulonii* and *C. vulturna* can cause false positives.

In conclusion, CHROMagar™ *Candida* Plus showed to be a welcome addition to the abundance of detection and identification methods for *C. auris*.

102C

Copper(II)-binding triazole derivatives: promising bifunctional antifungals.

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Abstract

The increasing incidence of invasive fungal infections (IFIs) is the result of many factors, including an increase in the resistance to current drugs, which is aggravated by an almost inexistent antifungal-drug development strategy over the last decade. There is as such an urgent need to obtain new drugs and/or antifungal strategies that are efficient, selective and able to overcome existing resistance mechanisms.

We have recently described the synthesis of a novel antifungal azole compound capable of chelating metals [1]. We showed that the organic compound **4** and its copper(II) complex **Cu.4** are active against *Candida albicans* and *Candida glabrata*. Importantly, both compounds are more effective than fluconazole against *Candida glabrata*, which is inherently very tolerant to azole drugs. We proposed that the ionophore activity, and consequent ROS production, of **Cu.4** is the basis of its antifungal action, while **4** acts by chelating the iron and depriving cells of this micronutrient. In line with the ionophore activity, blocking cellular copper detoxification increased susceptibility of *Aspergillus fumigatus*, against compound **Cu.4**. Unfortunately, the **Cu.4** complex was also highly toxic to mammalian cells, which strongly limits a potential medical application in its present form. We are currently developing alternative azoles containing metal chelating moieties to enable ergosterol inhibition. Such compounds are expected to reduce mammalian cytotoxicity, while increasing fungal toxicity.

[1] Gaspar-Cordeiro, A., et al. A copper(II)-binding triazole derivative with ionophore properties is active against Candida spp..J Biol Inorg Chem 25, 1117–1128 (2020). https://doi.org/10.1007/s00775-020-01828-6

103A

Liposomes functionalized with fungi-targeting peptide demonstrate increased interaction with Candida albicans

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Abstract

Candida albicans infections can be challenging to treat, as current antifungal drugs exhibit poor water solubility and host toxicity. To overcome these issues, new methods of drug delivery are needed. Liposomes have been shown to be an effective method for administrating antifungals and can increase bioavailability and solubility while decreasing toxicity. However, existing antifungal liposomal formulations lack infection specificity. For example, AmBisome, a liposomal formulation of amphotericin B, relies on passive accumulation to infection sites. We have developed antifungal liposomes that display fungi-targeting moieties to promote interaction with *Candida;* we predict that these formulations can increase fungal eradication and decrease off-site toxicity. Here, the *C. albicans*-targeting peptide P-113Q2.10 (AQRHHGYKRQFH), a derivative of the antifungal peptide histatin 5, was incorporated into liposomes via conjugation to palmitic acid (PA). PA-P-113Q2.10 conjugates were synthesized using solid phase peptide synthesis, confirmed by liquid-chromatography-mass spectrometry. Liposomes composed of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine and cholesterol with 1% w/w PA-P-113Q2.10 were formed via thin film-hydration and extrusion, yielding ~100 nm liposomes with a polydispersity index of ~0.1. Flow cytometry demonstrated that interaction with *C. albicans* SC5314 was enhanced for P-113Q2.10 liposomes, increasing from ~60% in cells incubated with liposomes lacking peptide to ~79%. These liposomes preferentially interact with *C. albicans* compared to NIH 3T3 murine fibroblasts; on average, only ~15% of fibroblasts incubated with liposomes (with and without peptide) showed positive liposome interaction. This liposome formulation has the potential to serve as an antifungal delivery platform that selectively targets *C. albicans* cells for increased efficacy in treatment of fungal infections.

104A

Candida albicans cell wall mannosidases, Dfg5 and Dcw1, regulate chitin synthesis

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Abstract

In *Candida albicans* chitin synthesis is important for cell wall integrity and may also have a role in emergence of drug-resistance. Our past studies showed that cell wall mannosidases, Dfg5 and Dcw1, regulate HOG MAPK signaling. In this study, we investigated how Dfg5 and Dcw1 regulate chitin synthesis by affecting HOG, PKC and Calcium-Calcineurin signaling pathways. *DFG5* and *DCW1* heterologous mutants (ES1 & ES195) and a conditional mutant (ES195+methionine/cysteine) were utilized. WT SC5314 served as negative control and Hog1 knock-out mutant as positive control. Fluorescence microscopy of calcofluor white (CFW) stained mutant and control strains was performed to observe chitin accumulation. Quantitative PCR analysis was performed to measure the relative expression of chitin synthases *CHS*1, *CHS2*, *CHS*3 and *CHS*8. Incubation with chitinase was done to determine cell separation using light microscopy and scanning electron microscopy (SEM) analysis. Fluorescence microscopy showed significantly increased chitin accumulation in the mutants as compared to wild type. Chitin accumulation was observed mainly at the budding sites indicating a cause for defective cell separation phenotype. Incubation with chitinase led to cell separation in the mutants. *CHS2, CHS3* and *CHS8* expression was observed to be significantly upregulated in the conditional mutant and *HOG1* mutant as compared to the wild type. This upregulation was also observed when the cell wall integrity PKC pathway was activated. However, activation of the Calcium-calcineurin pathway downregulated chitin synthase expression in the mutants. Our data indicates that Dfg5 and Dcw1 regulate expression of chitin synthases via HOG MAPK, PKC and Calcium-calcineurin signaling pathways.

105B

Multi-genic changes in chromosome 2 bypass the requirement for cAMP in *Candida albicans* hyphal growth

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Abstract

The ability to reversibly switch growth patterns between budding yeasts and filamentous hyphal cells is a distinctive feature of *Candida albicans* compared to most other fungi. Hyphal growth is linked to virulence properties as long hyphal filaments promote invasion into tissues and biofilm formation. Adenylyl cyclase (Cyr1) has long been thought to be the master regulator of hyphal growth, through activation of cAMP signaling. However, it is difficult to define the roles of the cAMP pathway because a *cyr1*Δ/Δ mutant grows very slowly and expresses abnormally low levels of genes needed for hyphal growth. Surprisingly, we discovered that faster growing *cyr1*Δ/Δ pseudorevertant (PR) mutants form hyphae in the absence of Cyr1 and cAMP. Genome sequence analysis identified multiple genetic changes in chromosome 2 that improve growth and hyphal induction of the PR mutants in the absence of adenylyl cyclase and cAMP. Genetic mapping by CRISPR-Cas9 indicated that the improved hyphal growth of the PR mutants is due to multiple genetic changes including heterozygosity of 3 genes involved in global transcription regulation. The PR and wild-type strains showed similar transcriptomic profiles during hyphal induction, indicating that cAMP is not necessary to activate hyphal genes during hyphal induction. Quantitative phosphoproteomics detected the activity of the key protein kinases that induce hyphae in *C. albicans*. This multi-omics analysis demonstrates that *C. albicans* can stimulate signal pathways to induce hyphae that are independent of adenylyl cyclase and cAMP.

106C

Candida albicans plasma membrane (PM) phosphatidylinositol-(4,5)-bisphosphate (PI(4,5)P_2) and septins interact to activate the PKC-Mkc cell wall integrity pathway, septation and cytokinesis

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Abstract

Background: We have shown that *C. albicans* PI(4,5)P_2 and septins interact to regulate PKC-Mkc1 cell wall integrity pathway, echinocandin and cell wall stress responses, and virulence during candidiasis. Our goal was to define PI(4,5)P_2-septin pathway interactions.

Methods: We created various CaGFP- and CaRFP-tagged reporters and used live cell imaging to localize pathway components.

Results: *C. albicans* exposure to caspofungin results in immediate PI(4,5)P_2, septin (Cdc10, Sep7), chitin, and cell wall protein (Rbt5) mislocalization to highly dynamic, aberrant PM foci at sites of arrested septation and cytokinesis, which resemble PM invaginations of *irs4* (EH domain protein), *inp51* (5'-phosphatase) and *gin4* (protein kinase) mutants with dysregulated PI(4,5)P_2. PI(4,5)P_2 is mislocalized in *mkc1* mutants in presence, but not absence of caspofungin. SC5314 PI(4,5)P_2 levels and PKC-Mkc activation increase in a dose-response manner at caspofungin concentrations ≤4× MIC and progressively decrease at concentrations ≥8× MIC. Caspofungin exposure results in broad-based mother-daughter bud necks and arrested septum-like structures, in which PI(4,5)P_2 and Cdc10 colocalize. PM invaginations in *irs4* and *inp51*, and in SC5314 exposed to caspofungin also mislocalize actinomyosin (Act1, Myo1). Finally, in response to caspofungin, PI(4,5)P_2 co-localizes with activated Rho1-GTP (as visualized using GFP fused to PKC's Rho1 binding domain) within PM foci and invaginations.

Conclusions: Our data support a model in which echinocandins and other stressors activate PKC-Rho1, resulting in PM PI(4,5)P_2-septin interactions that recruit actinomyosin and regulate cell wall integrity responses, septation and cytokinesis.

108B

Characterization of a *Candida albicans* mutant defective in all MAPKs highlights the major role of Hog1 in the MAPK signaling network

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Abstract

Candida albicans success as a fungal pathogen relies on its ability to adapt and proliferate in different environmental niches. Pathways regulated by mitogen activated protein kinases (MAPKs) are in charge of sensing and signal transduction, acting as a fungal nervous system to develop an accurate transcriptional response and circumvent inhospitable conditions. We have generated by sequential deletion of the four described MAPKs in *C. albicans* a mutant defective in all known MAPKs, Cek1, Cek2, Mkc1 and Hog1. This strain is viable and has no apparent defects in fitness under standard conditions. However, it is highly sensitive to stresses, both oxidative and osmotic and display a specific pattern of sensitivity to antifungals. By comparing its phenotype with single, double and triple combinations of MAPKs mutants we were able to unveil a Cek1-independent mechanism for *hog1* resistance to Congo red, as well as to confirm the predominant effect that Hog1 displays on oxidative and osmotic adaptation. The quadruple mutant produces filaments under non-inducing conditions but is unable to develop chlamydospores, therefore resembling the *hog1* phenotype. Furthermore, *cek1 cek2 mkc1 hog1* cells switch to the opaque state at high frequency, which is blocked by the ectopic expression of *HOG1*. The use of combinatorial MAPKs deletions not only allow the analysis of putative cross-talks between pathways but may also constitute an interesting tool in different areas of research due to its presumed lack of virulence.

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110A

Dissecting the circuitry governing temperature-dependent morphogenesis in *Candida albicans.*

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Abstract

Fungal diseases impose a significant burden on human health, killing at least as many people as malaria and tuberculosis each year. To establish infection in humans, fungi must be able to grow at mammalian body temperature, and this requirement is only met by a small subset of species within the fungal kingdom. One such species is *Candida albicans*, a leading human fungal pathogen which can cause life-threatening infections in immunocompromised individuals. The inextricable link between temperature and virulence becomes even more prominent in *C. albicans* through the impact of temperature on morphogenesis. The capacity to transition from a yeast to filamentous growth state is critical for pathogenicity and is induced by a number of cues, many of which require a minimum temperature of 37^{o}C to induce filamentation. However, the genetic circuitry through which temperature influences morphogenesis in *C. albicans* remains enigmatic. To explore this circuitry, I screened a mutant collection covering ~40% of the *C. albicans* genome and identified 23 genes required for morphogenesis at the elevated temperature of 39^{o}C. Interestingly, this analysis suggested a key role for mRNA splicing via the spliceosome. Furthermore, five of the genes identified were specifically required for filamentation in response to cues that require a temperature of 37^{o}C to induce morphogenesis, but were dispensable in response to other cues, highlighting a specific set of genes important for sensing and responding to elevated temperature. Overall, this work illuminates genes important for morphogenesis in response to high temperature, with implications for understanding *C. albicans* pathogenicity.

111B

Induction of protective mitochondria-associated type I interferon signalling and a damage-driven response in human vaginal epithelial cells during *Candida* infection

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Abstract

Vaginal candidiasis is a highly prevalent infection predominantly caused by *Candida albicans, C. glabrata C. parapsilosis,* and *C. tropicalis*. Using dual RNA-Sequencing, we dissected host-*Candida* interactions during infection of vaginal epithelial cells. The four most common *Candida* species exhibited distinct pathogenicity patterns, defined by species-specific transcriptional profiles. This suggests that different strategies to infect epithelial cells are the result of independent evolution of pathogenicity in these fungi. In contrast, the host response at early stages of infections was common to all species, and was characterized by protective type I interferon signalling induced *via* transient mitochondrial dysfunction. At later stages, the host response diverged in a species-dependent manner, which was primarily driven by a different extent of epithelial damage caused by each species. Our data show species-specific fungal transcriptional patterns and a biphasic host response characterized by a common early response and divergent damage-related responses at the later stages of infection. Moreover, we discovered a common epithelial type I interferon response to early host-*Candida* interactions, which mediates protection of epithelial cells against fungal-induced damage and modulation innate antifungal immune responses.

112C

Analysis of *Candida albicans* Cdk8 dependent phosphoproteome reveals a repression of hyphal growth through a Flo8 dependent pathway

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Abstract

Ssn3, also known as Cdk8, is a member of the four protein Cdk8 submodule within the multi-subunit Mediator complex involved in the co-regulation of transcription. In *Candida albicans*, like in other fungi, the loss of Ssn3 kinase activity affects multiple phenotypes and enhances hyphal growth. In these studies, we generated a strain in which Ssn3 was replaced with functional variant of Ssn3 that can be rapidly and selectively inhibited by the ATP analog 3-MB-PP1. Consistent with *ssn3* null mutant and kinase dead phenotypes, inhibition of Ssn3 kinase activity promoted hypha formation. Furthermore, the increased expression of hypha-specific genes was the strongest transcriptional signal upon inhibition of Ssn3 in transcriptomics analysis. Phosphoproteomics studies were performed to identify Ssn3 kinase substrates associated with filamentation potential. Protein phosphorylation sites that were reduced specifically upon Ssn3 inhibition, included two sites in Flo8, a transcription factor known to positively regulate *C. albicans* morphology. Mutation of the two Flo8 phosphosites (threonine 589 and serine 620) was sufficient to increase Flo8-HA levels and Flo8 dependent activity, suggesting that Ssn3 kinase activity negatively regulates Flo8. Lastly, our data suggests that Flo8 participates in Ssn3 regulation of metabolism, which has been described to control morphology.

113A

Candida albicans Sap6 activates protease activated receptor PAR2 to mediate p38 MAPK activation, IL-8 release and barrier break in oral epithelial cells

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Abstract

Hyphal-specific Secreted Aspartyl Proteases (Sap) modulate *C. albicans* pathogenicity. Higher levels of secreted Sap6 increase fungal virulence in murine oral candidiasis by increasing adhesion and invasion to oral epithelial cells (OECs). Purified *C. albicans* Sap6 activated production and secretion of proinflammatory cytokines and MAPK signaling in human monocytes promoted by protease activated receptors, however it is not known whether OECs respond to Sap6 in a like manner.

Objective: Determine OEC receptors that engage *C. albicans* Sap6 to produce inflammatory cytokines.

Results: Cytokine Profile array analyses of OECs treated with *C. albicans* (Ca) or recombinant Sap6 (rSap6) revealed selective induction of IL-1β and IL-8 cytokines. Western blotting of OECs lysates exposed to rSap6 or *Ca* hyphal cells showed increased phosphorylation of p38, MKP1 and c-Fos that was not found in cells exposed to *C. albicans Δsap6* mutants or heat-inactivated Sap6, suggesting PAR2 mediated signaling. Pretreating OECs with a PAR2 antagonist (FSLLRY-NH_2) prior to stimulation with Ca or Sap6 significantly reduced levels of p-38 phosphorylation, c-Fos and IL-8 secretion. This effect was specific to PAR2, as PAR1 antagonists did not affect Sap6-mediated OEC production of IL-8. Pretreatment of OECs with PAR2 antagonists reduced invasion of OECS by Ca and prevented degradation of E-cadherin and occludin junctional proteins associated with Ca invasion.

Conclusions: Sap6 induced p38 phosphorylation and IL-8 release in OECS that requires PAR2. Blocking PAR2 signaling protected OECs from epithelial barrier break suggesting that PAR2 inhibitors may be a potential treatment to prevent Ca epithelial invasion and damage.

114B

Dectin-1 Molecular Aggregation and Signaling is Sensitive to β-Glucan Structure and Glucan Exposure on *Candida albicans* Cell Walls

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Abstract

Dectin-1A is a C-type Lectin innate immunoreceptor that recognizes β-(1,3;1,6)-glucan, a structural component of *Candida* species cell walls. of β-glucans can adopt solution structures ranging from random coil to insoluble fiber due to tertiary (helical) and quaternary structure. Fungal β-glucans of medium and high molecular weight (MMW, HMW) are highly structured, but low MW glucan (LMW) is much less structured. Despite similar affinity for Dectin-1, the ability of glucans to induce Dectin-1A-mediated signaling correlates with degree of structure. Glucan denaturation experiments showed that glucan structure determines agonistic potential, but not receptor binding affinity. We explored the impact of glucan structure on molecular aggregation of Dectin-1A. Stimulation with glucan signaling decreased Dectin-1A diffusion coefficient in inverse proportion to glucan structural content, consistent with induced Dectin-1A aggregation. Fluorescence measurements provided direct evidence of ligation-induced Dectin-1A aggregation into dimers and small oligomer states, which positively correlated with increasing glucan structure content. In contrast, Dectin-1A is predominantly in a monomeric state in resting cells. Molecular aggregates formed during interaction with highly structured, agonistic glucans did not exceed relatively small (<15 nm) clusters of a few engaged receptors. Finally, we observed increased molecular aggregation of Dectin-1A at fungal particle contact sites in a manner that positively correlated with the degree of exposed glucan on the particle surface. These results indicate that Dectin-1A senses the solution conformation of β-glucans through their varying ability to drive receptor dimer/oligomer formation and activation of membrane proximal signaling events.

115C

Inter-subject variations and inter-*Candida albicans* dependence of human cellular immune responses in vitro

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Abstract

Based on an *in vitro* co-culture model of human PBMCs from healthy subjects with *Candida albicans*, we aimed at the long-standing question of inter-subject variation and inter-*C. albicans* dependence of the host immune response. We showed that *in vitro* immune responses against *C. albicans* strains variate among and within the subjects over the time. Moreover, we observed that host responses also depend on *C. albicans* strain used for the challenge. Through a combination of multiple fungal growth and flow cytometric measurements, coupled to the tSNE algorithm, we showed that significant proliferation differences exist among *C. albicans* isolates, leading to the calculation of a strain specific persistent index. Despite substantial inter-subject differences in T cells and stability of myeloid cells at baseline, our experimental approach highlights substantial immune cell composition changes and cytokine secretion profiles after *C. albicans* challenge. Fungal clearance was associated to the significant secretion of IL-17 by CD66+ cells, IFN-γ and IL-10 by CD4+ T cells 2 days after *C. albicans* challenge. Fungal persistence was associated with a delayed secretion of IFN-γ, IL-17, IL-4, TNF-α and IL-10 by myeloid cells and IL-4 and TNF-α secretion by CD4+ and CD8+ T cells. Overall, this experimental and analytical approach is available for the monitoring of such fungal and human immune responses.

116A

Oral epithelial IL-22/STAT3 signaling licenses IL-17-mediated immunity to oral mucosal candidiasis

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Abstract

Oropharyngeal candidiasis (OPC, oral thrush) is an opportunistic infection caused by the commensal fungus *Candida albicans*. IL-17 and IL-22 are cytokines produced by Type 17 lymphocytes. Both cytokines mediate antifungal immunity yet activate quite distinct downstream signaling pathways. While much is now understood about how IL-17 promotes immunity in OPC, the activities of IL-22 are far less well delineated. We show that, despite having similar requirements for induction from Type 17 cells, IL-22 and IL-17 function non-redundantly during OPC. We find that the IL-22 and IL-17 receptors are required in anatomically distinct locations within the oral mucosa; loss of IL-22RA1 or STAT3 in the oral basal epithelial layer (BEL) causes susceptibility to OPC, whereas IL-17RA is needed in the suprabasal epithelial layer (SEL). Transcriptional profiling of the tongue linked IL-22/STAT3 to oral epithelial cell proliferation and survival, but also, unexpectedly, to driving an IL-17-specific gene signature. We show that IL-22 mediates regenerative signals on the BEL that replenish the IL-17RA-expressing SEL, thereby restoring the ability of the oral epithelium to respond to IL-17 and thus to mediate antifungal events. Consequently, IL-22 signaling in BEL 'licenses' IL-17 signaling in the oral mucosa, revealing spatially distinct yet cooperative activities of IL-22 and IL-17 in oral candidiasis.

117A

Genotypic and Phenotypic Portrait of *Candida albicans* Clinical Isolates Colonizing the Airways of Patients with Cystic Fibrosis

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Abstract

Candida albicans colonizes the respiratory tract of patients with Cystic Fibrosis (CF). It competes with CF-associated pathogens, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and contributes to disease severity. We serially recovered 160 *C. albicans* clinical isolates over a period of 30 months from the sputum of 23 pediatric and 2 adult antifungal-naive CF patients at Children's Hospital Tunis and characterized the genotype and phenotype of a subset of strains using multilocus sequence typing (MLST) and growth assays on multiple stress-, filamentous growth- and biofilm-inducing media. Out of 16 patients regularly sampled for at least 9 months, 8 and 4 were chronically and transiently colonized with *C. albicans*, respectively. MLST analyses of 56 strains originating from 15 patients indicated that each patient was colonized with a single strain, while 8 patients (53%) carried isolates from clade 4 known to be enriched with strains from Middle East-Africa. A subset of these isolates with the same sequence type and colonizing 3 unrelated patients displayed altered susceptibility to cell wall-perturbing agents, suggesting changes in cell wall structure/function during growth in the CF lung. We also observed differential ability to filament and/or form biofilms in a set of identical isolates from clade 10 sampled over a period of 9 months in a pediatric CF patient, suggesting alterations in phenotypes associated with virulence. Our findings will rely on future whole-genome sequencing analyses to identify polymorphisms that could explain the emergence of new traits in *C. albicans* strains thriving in the CF host environment.

118B

Identification and Evolutionary Analysis of Putative Adhesin Gene Families in *Candida auris*

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Abstract

Candida auris is an emerging multidrug-resistant fungus. Among its pathogenic traits is its ability to strongly adhere to and persist on surfaces, making C. auris extremely difficult to eliminate once it establishes itself in healthcare facilities. Adhesin genes have been identified and shown to play a major role in the pathogenesis of another Candida pathogen, C. albicans, but the ALS family that was expanded in the C. albicans is not over-represented in the sequenced C. auris genomes (Muñoz et al. 2018), raising the question of whether novel adhesin genes exist in this species. Using candidate-gene based and genome-wide approaches to address this question, we identified a protein family likely to encode functional adhesins in C. auris. Phylogenetic analyses revealed that this family and its Pfam domain are fungal-specific, and that the family has expanded in parallel in the multi-drug resistant (MDR) clade containing C. auris and in the C. albicans and its close relatives. Interestingly, fold-based structure predictions for the N-terminal domain (NTD) from multiple members in the family suggest conformational similarities to unrelated bacterial adhesins containing the beta-solenoid fold, suggesting convergent evolution. The non-NTD portion of the protein homologs show rapid evolution, with the MDR clade homologs harboring a distinct pattern of repeat structure that contains a strong β-aggregation sequence motif. Together, our results reveal novel candidate adhesin genes in C. auris and its Clavispora relatives and identify key sequence features that are conserved and rapidly evolving.

119C

Identification of mechanisms regulating Candida albicans stress-induced genome instability.

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Abstract

Candida albicans is a commensal harmless organism that colonizes the majority of the population. However, it can become a life-threatening pathogen causing systemic infections that are fatal in ~50% of cases. During colonization and infection, *C. albicans* encounters many different host environments to which it must adapt rapidly. This lifestyle gives rise to a key question: “How does *C. albicans* respond rapidly to the continuous changes in the environment it encounters in the host?”

In recent years, the importance of stress-induced genome instability has emerged as a key adaptive mechanism of human fungal pathogens. Although excessive genome instability is harmful, moderate genome instability facilitates rapid adaptation to environmental insults. This is because genomic instability can increase genetic diversity, thereby allowing selection of genotype(s) better adapted in a new environment. The drivers and regulatory mechanisms underlying *C. albicans* stress-induced genome instability are largely unknown. Our hypothesis is that replicative stress, controlled by specific DNA damage pathways drives genome instability in stress environments. The goal of this project is to identify novel factors regulating DNA damage and stress-induced genome instability in *C. albicans*. We performed different parallel genetic screens using a homozygous *C. albicans* gene deletion library[1]. This approach has led to the identification of several *C. albicans* genes important for sensing and repair different types of DNA lesions. Our focus is to understand whether and how these novel factors regulate stress-induced genome instability and host adaptation.

1. Noble, S. M., French, S., Kohn, L. A., & Alexander, D. HHS Public Access. **42**, 590–598 (2011).

120A

Candida glabrata evolution towards posaconazole resistance: novel molecular insights

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Abstract

Candida glabrata is the second most common agent of candidiasis worldwide and its increasing prevalence is mostly related to its ability to rapidly develop azole resistance. The central mechanism of acquired azole resistance is the upregulation of drug efflux pumps due to the emergence of GOF mutations in the transcription factor *PDR1*. Posaconazole has the broadest antifungal spectrum among available triazole antifungals. Notably, the development of resistance is unwell characterized.

In this work, *in vitro* directed evolution was used to stress three azole susceptible clinical isolates towards posaconazole resistance, through prolonged exposure to clinical serum concentrations of posaconazole; resistance was reached following 10-25 days of incubation. Remarkably, no changes in the sequence of the *PDR1* or *ERG11* genes were found, suggesting the presence of unknown mechanisms underlying resistance development. To understand the origin of this resistant phenotype, the whole genome of the resistant isolates was sequenced, in the search for common mutated genes, when compared to the susceptible parental isolates. Five genes were found to display non-synonymous mutations in the three resistant isolates, when compared to the susceptible counterpart. These genes included 4 adhesin-like proteins and the Hxt4/6/7 putative hexose transporter. Interestingly, the expression of the *HXT4/6/7* gene was found to confer azole susceptibility. Consistent with the previous finding that azole drugs are imported through energy-independent facilitated transport into the yeast cell, our results suggest that Hxt4/6/7 may play a role in the uptake of azole drugs.

121B

High-frequency alterations within the *Candida albicans* genome promote adaptation to the gastrointestinal tract

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Abstract

Background: *Candida albicans* can infect diverse niches in the mammalian host due, in part, to its ability to adopt alternative phenotypic forms. A subset of clinical isolates had previously been observed to undergo phenotypic switching from the conventional “white” state to the “gray” state, although the mechanism of switching was unknown.

Methods: We analyzed the genome sequences and phenotypic properties of a number of clinical isolates of *C. albicans*. This included the use chromogenic CHROMagar to distinguish related phenotypic states such as white, gray and opaque. We examined clinical and engineered strains in both a commensal model of gastrointestinal colonization and a systemic model of candidiasis.

Results: We show that the white-to-gray transition is observed in strains that are functionally heterozygous for the *EFG1* transcription factor gene, and occurs due to loss of the single functional *EFG1* allele. The loss of *EFG1* is due to *de novo* mutation or gene conversion events that disrupt the *EFG1* locus. We further show that *EFG1* heterozygous strains readily lose functional *EFG1* during commensal colonization to become *efg1* null, and that these mutant forms then sweep the population. Similar results were found for strains heterozygous for the related transcription factor *FLO8*; these also became *flo8* null in the gastrointestinal tract where they similarly exhibited a fitness advantage.

Conclusions: We reveal that high-frequency genotypic changes can underlie common phenotypic transitions in *C. albicans*. In particular, heterozygous polymorphisms at target loci can readily become homozygous null mutants that enable adaptation to specific host niches.

122C

Global Translational Profile of the *Candida albicans* Morphological Transition

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Abstract

While several previous studies have examined the transcriptional profile of the *Candida albicans* yeast-filament transition, very little is known about translational mechanisms that are important for controlling morphology and other virulence-related processes in human fungal pathogens. Using a powerful genome-wide approach, ribosome profiling, we provide the first report of the global translational profile associated with the *C. albicans* morphological transition in response to growth in serum at 37°C. We have identified 176 genes showing significantly increased and 111 genes showing significantly reduced translational efficiency (TE) during filamentation. Strikingly, many genes involved in pathogenesis, response to stress and filamentation show reduced TE. Several of these genes are known to be strongly induced at the transcriptional level, suggesting that a translational fine-tuning mechanism may be in place. Using a recently developed and powerful ORF-calling method, we have identified over 1,200 potential uORFs in *C. albicans*, several of which are associated with genes involved in pathogenesis, and 57 potential novel ORFs, several of which show altered TE during filamentation. In addition, using a novel bioinformatics method for global analysis of ribosome pausing that will be applicable to a wide variety of genetic systems, we demonstrate that *C. albicans* genes involved in protein synthesis and cell wall functions are associated with an enrichment in ribosome pausing sites. Altogether, our results suggest that the *C. albicans* morphological transition is associated with widespread global translational alterations that do not simply reflect transcriptional changes and affect the expression of many genes involved in virulence-related processes and pathogenesis.

123A

Hypo-osmotic stress underlies general cellular defects of aneuploidy in yeast

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Abstract

Large-scale genome instability, such as aneuploidy, confers phenotypic plasticity and is evidently linked with antifungal resistance in pathogenic fungi. While aneuploidy represents thousands of random chromosome stoichiometry, diverse phenotypic profiles from individual aneuploid cells impede the development of therapeutic and diagnostic strategies against antifungal resistance. Here we analyzed genomic expression patterns in aneuploid populations harboring random aneuploid karyotypes using non-pathogenic yeast *Saccharomyces cerevisiae* and identified transcriptomic signatures that are suggestive of hypo-osmotic stress responses irrespective of their genome contents. This biophysical signature, hypo-osmotic stress in aneuploidy results from the proteome imbalance due to massive gene copy number variations, and a unique ploidy-cell size relationship in aneuploidy can be predicted by a thermodynamics modeling. Hyper-activation of MAP kinase also indicates cell wall stress caused by increased intracellular turgor pressure in aneuploid cells. Consequently, cytoplasmic environment and cell surface dynamics are altered in response to stress, while endocytosis is defective in aneuploidy. Nutrient homeostasis, such as concentrations of intracellular amino acids is also remodelled, as an indicative of metabolic stress. We further examined the stress responses of aneuploid cells under a thermal ramping condition and found delayed presence of protein aggregates in aneuploid cells, suggesting the aneuploid state may promote stress tolerance. In conclusion, the hypo-osmotic stress state is a common biophysical property in aneuploid cells that may provide a distinct cellular environment for stress adaptation. We are now leveraging these information from the model yeast to study the adaptive mechanisms in *Candida* species with ongoing large-scale genome instability.

124B

The Genome of Candida africana reveals structural variations compared to C. albicans

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Abstract

C. africana is considered a taxonomic variant of *C. albicans* belonging to clade 13 of the *C. albicans* complex. It lacks the ability to produce chlamydospores and also unable to assimilate N-acetylglucosamine and glucosamine. Actually a full genome of *Candida africana* is lacking, so the aim of this study was to assemble for the first time the haploid genome of *C. africana.* Raw illumina Hiseq data were downloaded from the European Nucleotide archive with the accession number SRR6669859. Adapters were removed using Trimmomatic (v.0.39). Remaining good quality reads were analyzed with kat (v.2.4.2) to investigate heterozigous ratio and than assembled with shovill (v.1.0.9) using a range of Kmer values. Homozygous genome was produced using redundans (v.0.14a). The resulting genome was mapped to the haploid reference *Candida albicans* SC5314_A22 strain using the D-GENIES webtool (http://dgenies.toulouse.inra.fr), and contigs were reordered based on the reference. Gapfilling (v1-10) was used for the gap closure step. The quality and completeness of the final genome was then evaluated using QUAST and BUSCO. Assemblytics (http://www.assemblytics.com) was used to determine structural variations between *C. africana* and *C. albicans,* after alignment using nucmer.

The *C. africana* sequences aligned well with the *C. albicans* reference genome used. The genome has 286 complete BUSCOs corresponding to 98.6% completeness with N50 of 2146907.

Structural variations detected by assemblytics showed a total of 331 variants affecting 270 kbp bases. A total of 114 insertions affecting 35454bp sequences were observed followed by This study forms foundation for the study of the impact of these variations on *Candida africana*.

125C

Candida auris Information Available at the *Candida* Genome Database

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Abstract

Candida auris is an emerging fungal pathogen that is becoming a major health concern globally, due to its multi-drug resistance and the ease with which it spreads patient-to-patient in hospital settings. To help facilitate research into this growing threat, we have added information for *Candida auris* strain B8441 to the *Candida* Genome Database (CGD: www.candidagenome.org). *C. auris* is the fifth *Candida*-related species for which CGD provides complete and up-to-date curation of the literature (in addition to *C. albicans* SC5314, *C. glabrata* CBS138, *C. dubliniensis* CD36, and *C. parapsilosis* CDC317). For each *C. auris* gene, we provide Gene Ontology (GO) annotations for gene product function, biochemical process, and subcellular location, based on published experimental data, or predicted from homology with well-characterized genes from other species. Gene and protein sequences are available for all CGD search and analysis tools, including BLAST. CGD's multiple sequence alignments and phylogenetic trees for orthologous groups of *Candida* genes have been updated to include *C. auris* orthologs. *C. auris* genome and annotation is available in the GBrowse and JBrowse Genome Browsers, and we will add large-scale experimental datasets to JBrowse as they become available. We welcome your feedback and suggestions, which can be sent to:

candida-curator@lists.stanford.edu.

This work was supported by the National Institute of Dental and Craniofacial Research at the US National Institutes of Health [grant no. R01 DE015873]

126A

Farnesol Secretion as a Possible Driving Force for Maintaining *Candida albicans* as a Diploid

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Abstract

Candida albicans is a pathogenic dimorphic fungus which is invariably found as a diploid in patients. *C. albicans* secretes the sesquiterpene farnesol both as a quorum sensing molecule which blocks the yeast to hypha conversion and as a virulence factor for pathogenicity. 20-25 µM farnesol kills other competing yeasts and fungi, often by triggering apoptosis, and yet wild type diploid *C. albicans* tolerates 300-500 µM farnesol. The recent availability of 10 haploid strains of *C. albicans* (5 mating type **a** and 5 mating type α) allowed us to compare their production of and sensitivity to farnesol. On average, the heterozygous diploid strains of *C. albicans* were 2.4 times more resistant to 20-40 µM farnesol than MTL**a** haploid cells and 4.6 times more resistant than MTLα haploid cells. Furthermore, the MTL**a** haploids produce approximately 10 times more farnesol than do the MTLα haploids. Prior work concluded that haploid strains exhibited such low fitness that *C. albicans* was thought to be an obligate diploid. We now suggest that increased farnesol secretion by the MTL**a** haploids and increased farnesol sensitivity of the MTLα haploids is a mechanism for maintaining the dominant heterozygous diploid status of *C. albicans*. This idea is based on the observation that the **a**-factor peptide pheromone is farnesylated but the α-factor pheromone is not farnesylated. Our working hypothesis is that farnesol is secreted in part via Ste6 and imported in part via Ste3, the proteins which export and import the farnesylated **a**-pheromone. We also examined whether farnesol was excreted in extracellular vesicles.

127B

Framework for connection of genotype to phenotype among Candida albicans isolates

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Abstract

Isolates of *Candida albicans* make up a genetically and phenotypically diverse population. However, the link between genotype and phenotype in these isolates remains unclear, meaning that there is limited predictive power one can garner from gene sequence data that will inform conclusions about a given isolate's biological traits. One key intermediary between genotype and phenotype is gene expression, and previous work has shown that there is significant regulatory network divergence between clinical isolates. Here we used RNA-seq analysis as well as phenotypic quantification of a panel of *C. albicans* isolates to draw connections between gene expression profiles and a phenotypic output. To do this we first assessed regulatory divergence by using a CRISPR-based approach to make null mutations of the key filamentation regulator, *EFG1*, in 17 clinical isolates. We then compared the phenotypic and regulatory impact of this mutation across strains to assess conservation of function of *EFG1* between strains which show similar transcriptional responses. We were thus able to establish a core *EFG1* network that is shared among all 17 strains as well as genes whose regulation was clade specific. The next question we addressed was whether the wild type isolates could be linked via gene expression according to their shared phenotypic traits. We will use these data to connect genotype to phenotype via gene expression and isolate candidate genes.

128C

Gene function uncovered via intra-species transcriptional profiling of Candida albicans

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Abstract

Background: Clinical isolates of *Candida albicans* display widespread genetic diversity and phenotypic variation for simple and complex traits including virulence. Significant challenges exist in linking the polymorphisms between strains responsible for different phenotypes that can be aided by transcriptional profiling of diverse isolates.

Methods: We performed RNA sequencing of a well-characterized set of 21 clinical isolates grown in rich medium at 30^{o}C. Gene expression was linked to 25 phenotypes through differential analysis, linear correlation, and assembly of co-expression modules by weighted gene correlation network analysis (WGCNA). Genes in modules associated with growth and filamentation differences among the clinical isolates were assessed by targeted disruption and phenotyping.

Results: Hierarchical clustering of RNA expression profiles did not reproduce isolate phylogenetic relationships. In fact, up to 40% of the genic repertoire was differentially expressed between any two strains, with strain-specific expression of 679 (of 6000) genes in one isolate. Association of gene expression and phenotypes among the 21 isolates revealed that phenotypic differences could be linked both to previously characterized and novel pathways. Furthermore, co-expression modules could be linked to simple and complex phenotypic traits, and experimental investigation of eight genes in two modules confirmed their predicted roles in growth and filamentation.

Conclusion: The extensive transcriptional diversity among *C. albicans* isolates can be used to resolve species-level and strain-specific transcriptional architecture. Experimental testing of several genes predicted to modulate *C. albicans* virulence-related phenotypes supports our approach, and reveals novel regulators of *C. albicans* phenotypes can be uncovered by transcriptional analysis of clinical isolates.

129A

Mechanisms of miltefosine resistance in *Candida parapsilosis*

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Abstract

Candida parapsilosis is a major cause of candidiasis, particularly in the very young and the very old. We carried out a detailed genomic analysis of 95 clinical and environmental isolates of C. parapsilosis, including several blood and fecal isolates from recipients of allogeneic hematopoietic cell transplants. We identified a high rate of Copy Number Variations (CNVs), particularly of the RTA3 open reading frame.

RTA3 encodes a putative floppase, and is hypothesised to regulate the asymmetric distribution of phosphatidylcholine across the membrane. We found that the copy number of RTA3 varied from two to >40 across multiple isolates. From 50 isolates with an amplification of the region, 11 distinct endpoint pairs were observed. For a number of isolates tested, the increased copy number of RTA3 correlated with resistance to miltefosine, a drug known to inhibit phosphatidylcholine biosynthesis. We propose that an unknown selective pressure leads to amplification of RTA3 in C. parapsilosis, resulting in drug resistance.

In addition, we conducted a lab evolution experiment where C. parapsilosis isolates were cultured in increasing concentrations of miltefosine over a 26-day period. None of the resulting miltefosine-resistant strains had an amplification at the RTA3 locus.However we identified two lipid transporter genes, CPAR2_303950 and CPAR2_102700, which have protein-affecting variants in the evolved strains. The CPAR2_303950 paralog in S. cerevisiae, DNF1, is known to have phosphatidylcholine flippase activity. These analyses point towards the regulation of phosphatidylcholine transport as a potential route to miltefosine resistance in C. parapsilosis.

130B

Evolution of biofilm formation in *Candida* species

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Abstract

The ability of *Candida* species to form biofilms is essential for the colonization of different niches in the human host. Biofilm formation by *C. albicans*, the clinically most important species, starts with adhesion of yeast cells to the surface, followed by filamentation to form the upper layers of the biofilm. The transcription circuit that regulates this process in *C. albicans* consists of seven core transcription regulators that control each other's expression and the expression of more than one sixth of the gene total. Despite the complexity of the circuit, there are several lines of evidence that suggest that recently it underwent considerable evolutionary change. To understand how biofilm formation came about in *Candida*, we characterized biofilm formation and the underlying transcription circuit in several related species. Our results show that only closely related species to *C. albicans* are able to form structured biofilms with a basal layer of yeast cells and an upper layer of hyphae. In agreement, the biofilm transcription circuit is considerably different in species that form simpler biofilms, including the core transcription regulators. However, most of the differences observed are due to changes in the target genes, even between closely related species that form similar structured biofilms. This changes in the target genes are mostly explained by modifications in cis-regulatory elements. Overall, our results suggest that this circuit does not evolve as a whole despite the high interconnectivity of its components; there is considerable turnover of individual transcription regulators and especially of target genes between species.

131C

Balancing positive and negative selection: *in vivo* evolution of *Candida lusitaniae MRR1*

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Abstract

The evolution of pathogens in response to selective pressures present during chronic infections, such as nutrient limitation and interaction with immune cells or other microbes, can influence persistence, virulence, and the outcomes of antimicrobial therapy. Our analysis of a set of clonally-derived *Clavispora (Candida) lusitaniae* isolates, from a fungus-dominant lung infection from an individual with cystic fibrosis, found striking genomic changes that appeared to repeatedly arise in this chronic infection. We observed an enrichment in the number of alleles of *MRR1* and genetic and genomic analyses found evidence for repeated acquisition of gain-of-function mutations that conferred constitutive Mrr1 activity. In the same population, there were multiple alleles with both gain-of-function mutations and secondary suppressor mutations that either attenuated or abolished the constitutive activity suggesting the presence of counteracting selective pressures. Our studies demonstrated tradeoffs between high Mrr1 activity, which confers resistance to the antifungal fluconazole, host factors, and bacterial products through its regulation of *MDR1*, and resistance to hydrogen peroxide, a reactive oxygen species produced in the neutrophilic environment associated with this infection. This inverse correlation between high Mrr1 activity and hydrogen peroxide resistance was observed in multiple *Candida* species, in serial analysis of populations from this individual collected over three years and was associated with broad metabolic changes. These data lead us to propose that dynamic or variable selective pressures can be reflected in population genomics and that these dynamics can complicate the drug resistance profile of the population.

132A

Deriving Accurate Sequences Encoding the Fungal Adhesinome

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Abstract

Fungal adhesion is a prerequisite for disease development. The availability of genome sequence data has advanced the study of fungal adhesion. However, genes encoding adhesins are often large, belong to families that have many highly similar loci, and encode long stretches of repeated DNA that are difficult to assemble especially from short-read sequence data. These circumstances are a barrier to a thorough understanding of fungal adhesins and their relatedness across fungal species. The goal of this work is to accurately sequence and annotate fungal adhesin-encoding genes. These data will bolster important genomic reference sequences and facilitate functional analysis of proteins with potential for adhesive function (i.e. constituents of the adhesinome). The project utilized existing genome sequences and generated novel sequences that incorporated data from emerging long-read sequence technology. Potential adhesin-encoding loci were identified, then PCR amplified. Sanger sequencing was used to assemble accurate genes. Work was completed for the agglutinin-like sequence (*ALS*) family. *ALS* genes were found in pathogenic and non-pathogenic species, with a range of 1 to 29 *ALS* loci among the species studied. The character of the Als adhesive domain defined in *Candida albicans* varied with increasing phylogenetic distance from this reference point. Accurate sequence data were leveraged to develop assays to quantify expression from each specific locus and identify genes likely to produce abundant cell-surface Als protein. Efforts are now focused on other adhesin families and singleton genes. Data from this study will advance efforts to understand and potentially disrupt interactions between fungal pathogens and the host.

133B

Phylogeny and ploidy variation of *Candida albicans* and *C. glabrata* clinical isolates from Manitoba, Canada

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Abstract

Candida is globally distributed, yet North American strains are underrepresented in global phylogenic studies. Furthermore, the majority of such studies have been based on multilocus sequence type (MLST) analysis that does not capture the important genomic characteristics that define *C. albicans*. Here we sought to determine the phylogenetic structure and further describe the ploidy variation, and fluconazole susceptibility of 43 *C. albicans* clinical strains from the Canadian province of Manitoba. A global approximate maximum likelihood whole-genome phylogeny revealed the strains are located throughout the global phylogeny but more clustered than expected by chance. We then conducted an *in-silico* MLST analysis to place the strains into the PubMLST database of 3600 profiles, which revealed the clusters are distributed in six clusters. Coverage analyses of reads mapped to each chromosome identified four whole chromosome aneuploidies in three strains and one ~115 kb duplication in one other strain. Using a disk diffusion assay, we found that all the strains were susceptible to fluconazole. We also measured drug tolerance; a trait distinct from resistance which has recently been shown to potentially influencing infection clearance. The isolates varied in tolerance to fluconazole much more than susceptibility, yet tolerance was not associated with either aneuploidy or phylogeny. Taken together, these preliminary findings indicate that regionally-acquired strains maintain a regional clustered structure even when placed in a global phylogenetic context. This is interesting as prior studies fail to find a strict link between phylogeny and geography; our future work will determine the temporal stability of this result.

135A

The genomic determinants of drug resistance in Candida glabrata

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Abstract

Candida fungal pathogens have become a major source of life-threatening agents over the last decades. There are very few families of antifungal drugs, and resistance towards them is increasingly reported, particularly for emerging species such as the yeast Candida glabrata. A key step towards solving the problem is understanding the molecular mechanisms of resistance, which are likely generated by adaptive mutations. We have investigated this by in vitro evolving Candida glabrata populations exposed to several of these drugs, followed by whole-genome sequencing. We have performed these experiments with fluconazole, anidulafungin and the serial combination of both, which mimic standard clinical therapy. Here, we report the genomic changes (including small mutations and structural variants) appearing in the evolution with each drug. In brief, we find mutational signatures of each treatment that are consistent with previous work in pathogenic yeasts. As an example, FKS1/FKS2 mutations are widely associated with anidulafungin resistance, while PDR1/ERG11 changes appear in fluconazole. In addition, we find a potential role of several structural variants, suggesting that the traditional focus on SNPs is underpowered to understand the genomic drivers of drug resistance. All in all, this work represents a comprehensive evaluation of the evolutionary processes that confer drug resistance to Candida glabrata.

136A

Mucosal IgA Prevents Commensal Candida albicans Dysbiosis in the Oral Cavity

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Abstract

The fungus *Candida albicans* colonizes the oral mucosal surface of 30–70% of healthy individuals. Due to local or systemic immunosuppression, this commensal fungus is able to proliferate resulting in oral disease, called oropharyngeal candidiasis (OPC). However, in healthy individuals *C. albicans* causes no harm. Unlike humans mice do not host *C. albicans* in their mycobiome. Thus, oral fungal challenge generates an acute immune response in a naive host. Therefore, we utilized *C. albicans* clinical isolates which are able to persist in the oral cavity without causing disease to analyze adaptive responses to oral fungal commensalism. We performed RNA sequencing to determine the transcriptional host response landscape during *C. albicans* colonization. Pathway analysis revealed an upregulation of adaptive host responses due to *C. albicans* oral persistence, including the upregulation of the immune network for IgA production. Fungal colonization increased cross-specific IgA levels in the saliva and the tongue, and IgA^+ cells migrated to foci of fungal colonization. Binding of IgA prevented fungal epithelial adhesion and invasion resulting in a dampened proinflammatory epithelial response. Besides $CD19^+$ $CD138^-$ B cells, plasmablasts, and plasma cells were enriched in the tongue of mice colonized with *C. albicans* suggesting a potential role of B lymphocytes during oral fungal colonization. B cell deficiency increased the oral fungal load without causing severe OPC. Thus, in the oral cavity B lymphocytes contribute to control commensal *C. albicans* carriage by secreting IgA at foci of colonization thereby preventing fungal dysbiosis.

137B

a1-antitrypsin augments host defense against C. albicans, but also induces hyphal formation

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Abstract

Antifungal resistance and high mortality highlight the need for novel therapeutic strategies to treat candidiasis. a1-antitrypsin (AAT), is an endogenous acute-phase protein that inhibits serine proteases and exhibits immune-modulating properties. Experimentally it was shown beneficial for anti-bacterial host defense. Moreover, AAT has life-saving benefits in graft versus host disease patients. We hypothesized that AAT could be a promising candidate for immunotherapy of fungal infections, and explored its potential using a murine systemic candidiasis model.

Despite a reduced kidney fungal burden in mice treated with plasma-derived human AAT, no improvement of candidiasis outcome was observed. To explore the underlying mechanisms, primary human immune cells (monocytes) were exposed to *C. albicans* and AAT in different concentrations. While, low AAT concentrations (1-10 mg/mL) enhanced phagocytosis and killing, high concentrations of AAT (1 mg/mL) negatively impacted *C. albicans* killing by monocytes. AAT further augmented hyphal growth accompanied with cell wall remodeling that improved immune recognition. In line with this, the killing of yeast-locked mutants was not improved by AAT. Specifically, we observed that AAT induced transcriptional changes in *C. albicans* that may account for the changes in pathogenicity.

The mammalian immune system has developed elegant strategies, such as AAT, to alter host defense against invading pathogens. However, our data suggest that besides the beneficial effects of AAT on specific host defense mechanisms, *C. albicans* can respond to AAT by modulating key virulence attributes such as hyphal growth. This suggests that *C. albicans* may have evolved mechanisms to sense host immune system proteins to efficiently induce counter-strategies.

138C

Neutrophil Swarming Delays the Growth of Pathogenic Fungi Clusters

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Abstract

Background

Neutrophils represent the largest population of white blood cells in the body. They play critical roles in antimicrobial defenses. Neutrophil swarming could be a key process for sealing off sites of infection and protecting healthy tissues. Emerging technologies allow the rigorous study of neutrophil swarming but, while neutrophils have long been appreciated as critical for defense against fungi, the role of neutrophil swarming during fungal infection remains unknown.

Methods

To directly examine the role of neutrophil swarming in the containment of microbes, we have developed a tool that enables the study of thousands of swarming. Neutrophil swarming is triggered on large arrays of clusters of microbes and timelapse observation of the swarms allows the detailed monitoring of microbe-neutrophil interactions.

Results

We tested the swarming of human neutropils on live fungal clusters. We found that human neutrophils swarmed vigorously against *C. albicans*. Swarms significantly delayed the growth of *C. albicans* hyphae for up to 16 hours. Interestingly, neutrophils neutralized other species like *C. glabrata* without the formation of significant swarms. Disruption of neutrophil function provided molecular insight into the antimicrobial mechanisms deployed against *C. albicans* during swarming. Intriguingly, we found that cytokine priming could directly enhance the ability of swarming to restrict and kill *C. albicans*.

Conclusions

Neutrophil swarming occurs against live fungi and contains the growth of *Candida* spp. Perturbations of swarming and NET formation enable *Candida* to escape the swarms. These results establish swarming as a potentially important mechanism of fungal control that warrants further investigation.

139A

Trained innate immune protection by Gr-1^{+} leukocytes against fungal sepsis induced by several routes of inoculation in mice vaccinated with low virulence *Candida* species

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Abstract

Background: We recently discovered a novel form of trained innate immunity (TII) induced by low virulence *Candida* species (i.e. *C. dubliniensis*) that protects against fungal/bacterial lethal mortality. Mice given a primary intraperitoneal (i.p.) challenge (vaccination) are protected against lethal sepsis originating from *C. albicans/Staphylococcus aureus* (*Ca/Sa*) intra-abdominal infection (IAI) or *Ca* bloodstream infection (BSI). The protection against IAI is mediated by long-lived Gr-1^{+} leukocytes as putative myeloid-derived suppressor cells (MDSCs) and not by prototypical trained macrophages. This study aimed to determine if a similar TII mechanism is protective against BSI and whether this TII can also be induced following intravenous (i.v.) vaccination. **Methods**: Mice were given primary challenge of low virulence *Candida* (i.p. or i.v.) followed by lethal challenge (*Ca/Sa* i.p. or *Ca* i.v.) 14 days later, and observed for sepsis (hypothermia, sepsis scoring, serum cytokines), organ fungal burden, and mortality. Similar parameters were monitored following depletion of macrophages or Gr-1^{+} leukocytes during lethal challenge. **Results:** Mice vaccinated i.p. or i.v. were protected against lethal *Ca/Sa* IAI or *Ca* BSI. In all cases protection was mediated Gr-1^{+} putative MDSCs with no role for macrophages, and correlated to reduced sepsis parameters. Protection also correlated to reduced fungal burden in spleen and brain, but not liver or kidney. **Conclusion:** These results suggest that Gr-1^{+} MDSC-mediated TII is induced by either i.p. and i.v. routes of primary challenge and protect against IAI or BSI forms of systemic candidiasis, with survival correlating with amelioration of sepsis and reduced organ-specific fungal burden.

140B

Candida auris cell wall mannans contribute to evasion of phagocytosis by neutrophils

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Abstract

Background: The globally emerging pathogen *Candida auris* resists phagocytosis by human neutrophils, leukocytes important for controlling invasive candidiasis. Little is known about why *C. auris* is not efficiently recognized and engulfed upon encounter with neutrophils. We hypothesized that outer cell wall mannoproteins may contribute to evasion by masking β-glucan.

Methods: We constructed *C. auris* mutants with disruption of predicted and putative mannan pathways by homologous recombination. We analyzed cell walls by TEM. Human neutrophils were isolated for examination of *Candida*-neutrophil interactions. We analyzed phagocytosis by fluorescence microscopy, fungal killing by viable plating, neutrophil extracellular trap formation (NET) formation by Sytox green fluorescence and scanning electron microscopy, and ROS production by CM-H2DCFDA fluorescence. To compare levels of exposed β-glucan, we labeled cells with recombinant murine Dectin-1 receptor and imaged with immunofluorescence microscopy.

Results: We found that neutrophils more rapidly engulfed *C. auris* mutants with disruption of *PMR1* or *VAN1*, nearly 3.5-fold and 2.5 fold, respectively, when compared to the parent strain. Both mutants were more susceptible to neutrophil killing and induced higher ROS levels than the parent strain. We did not observe significant differences in NET formation in response to the parent and mutant strains. Both *pmr1Δ* and *van1Δ* exhibited greater cell surface β-glucan. Complementation of *VAN1* in *van1Δ* reversed the phenotypes.

Conclusion: Disruption of cell wall mannan pathways in *C. auris* augments phagocytosis and enhances the killing by neutrophils. The findings suggest that *C. auris* cell wall mannans function to mask β-glucan, impairing innate immune responses.

141C

Defining heterogeneous neutrophil populations that respond to *Candida albicans*

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Abstract

Neutropenia is a major risk factor for developing invasive candidiasis, so identifying neutrophils control fungal pathogens is important. While once viewed as a uniform population, neutrophils have been proposed to exist as a heterogeneous population, with subsets which may be better able to respond to different stimuli like cancer, infection, or injury. We are interested in determining whether neutrophils exist as distinct populations or are predisposed to skewing into a certain phenotype; some which may be better able to respond to fungal pathogens. To test this hypothesis, we generated conditionally-immortalized single cell clones of the ER-Hoxb8 granulocyte monocyte progenitor (GMP) cell line. Cells from each clone were matured into neutrophils, then systematically tested for phagocytosis, killing ability, and ROS production in response to *Candida albicans*. We observed different responses between clones, suggesting that neutrophils may be predisposed to respond to different conditions, and some subpopulations of neutrophils may be better able to respond to fungal pathogens than others. Additionally, clonal GMPs that are reintroduced into a murine host mature into functional neutrophils and respond to *Candida albicans* challenge similarly. Together, this data indicates that neutrophils may not be a homogenous population. Instead, these data suggest that they may consist of subpopulations with different predetermined responses, responses that could render individual neutrophils better or worse suited for responding to different stimuli. By further characterizing these subpopulations, we may be able to leverage this during infection or inflammation by promoting one population of neutrophils over another to clear infection or reduce inflammation.

142A

The Effect of PAD4-deficiency Against Systemic *Candida albicans*.

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Abstract

Problem: Systemic candidiasis continues to be a significant problem in critically ill patients with high morbidity and mortality despite available antifungal therapy. We showed that human neutrophils produce Neutrophil Extracellular Traps (NETs) that compromise Candida hyphal viability *in vitro* suggesting that NETs can be an effective component in anti-fungal protection. Peptidylarginine deiminase 4 (PAD4) is required for chromatin disassembly which is essential for NETosis so that PAD4 inhibition or deletion is sufficient to abrogate NET release in neutrophils. However, controversy exists regarding the value of NET release to overall host defense *in vivo*, therefore we tested the hypothesis that the elimination of NET release (by using mice genetically depleted for PAD4) would result in a significantly diminished immune response to systemic candidiasis. Methods: WT and PAD4 knockout mice were challenged with an intravenous injection of 1x10^5 C. albicans (SC5314, ATCC). Mice were sacrificed at day 1, 2, 3, 4 and 5 post injection for histological examination, and quantification of fungal burden (quantified by colony forming units, CFU). Results: PAD4 deficiency resulted in a significant INCREASE in kidney fungal burden compared to wildtype controls at days 1 through 4 post systemic *Candida* challenge. Conversely, PAD4 deficiency resulted in a significant DECREASE in kidney fungal burden compared to wildtype controls at day 5 post systemic Candida challenge. Conclusions: This study demonstrates PAD4 activity is required at early timepoints in defense against systemic Candidiasis, while PAD4-independent mechanisms can eventually compensate resulting in successful anti-microbial response.

143B

Cross-protection against Experimental Systemic Candidiasis by Using a Murine Interferon Gamma-Producing Cryptococcus neoformans strain

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Abstract

Candida albicans is the most common etiological agent of candidiasis, and invasive candidiasis carries high levels of mortality despite currently available therapy. To date, there is no approved vaccine available for candidiasis. Our group has previously demonstrated that vaccination with a *C. neoformans* serotype A strain engineered to produce interferon-γ (strain H99γ) confers protection against experimental cryptococcosis in mice. Here we have used this same *C. neoformans* H99γ strain to immunize BALB/c mice in order to determine levels of cross-protection in a murine model of hematogenously disseminated *C. albicans* infection. Results indicated significant levels of protection in mice vaccinated with the H99γ strain as compared to a control group of mice immunized with heat-killed H99γ (HKH99γ), as determined by both survival rates and fungal tissue burden. Compared to HKH99γ-immunized mice, cytokine analysis of kidney homogenates from H99γ-immunized mice on day 3 post-challenge revealed increased levels of IL-17A, RANTES, IL-2 and IL-12(p40). Histopathological studies revealed that filamentous lesions in kidneys were much more prominent in HKH99γ-immunized mice as compared to those animals actively vaccinated with the H99γ strain, with H&E staining confirming the early recruitment of a high number of neutrophils at the site of infection in H99γ-immunized kidney tissue. In conclusion, our study indicates that significant levels of cross-protection against invasive candidiasis can be achieved by vaccination with an interferon-γ producing *C. neoformans* strain, and provides further insights towards the development of a potential pan fungal vaccine.

144C

Modulation of Mucosal Th2 Immunity in a Murine Model of IgE-Mediated Food Allergy

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Abstract

IgE-mediated food allergies are multifactoral diseases in which allergen exposure, host genetics, microbiome composition and environment combine to modulate disease susceptibility and progression. Using a previously described murine model of food allergy to egg, we investigated the impact of gastrointestinal (GI) colonization by the yeast *Candida albicans* on Th2 immunity and the severity of the anaphylactic response to ovalbumin (OVA) in sensitized mice. Both "responder" Balb/c and "non-responder" C57BL/6 mice were studied. Mice were given an oral gavage of *C. albicans* CHN1 during a 7 day treatment with amoxicillin, followed by OVA immunization and oral challenge. Colonization levels of *C. albicans* were roughly equivalent in both strains of mice and unaffected by the development of a mucosal Th2 response. Balb/c mice developed very robust Th2 immunity in the GI tract and an anaphylactic response following OVA sensitization and challenge. Colonization by *C. albicans* increased the number of mast cells in the GI tract and increased the incidence of diarrhea in Balb/c mice. In contrast, C57BL/6 mice did not develop very strong Th2 immunity and did not exhibit signs of anaphylaxis after sensitization and oral challenge with OVA. However, *C. albicans* colonization in these mice increased OVA-specific IgE levels in the serum, intestinal mast cell numbers and intestinal expression of a number of cytokines, including IL-9. These data support the idea that including GI colonization by *C. albicans* has the potential to modulate mucosal immunity to food-borne antigens.

145A

Candida-induced changes in the bone marrow compartment direct trained innate immune protection against fungal/bacterial sepsis

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Abstract

Background: Our laboratory established a clinically relevant mouse model of intra-abdominal infection (IAI) with *Candida albicans* and *Staphylococcus aureus* that results in acute lethal sepsis. Prior inoculation with low virulence *Candida* species results in 80-100% protection against the lethal sepsis. Protection is dependent on long-lived Gr-1^{+} myeloid-derived suppressor cells (MDSCs) as a novel form of trained innate immunity. Surprisingly, low virulence *Candida* species were found to penetrate the femoral bone marrow (BM) within 24 h of intra-abdominal exposure with the level of protection correlating to fungal BM burden. Our objective was to interrogate *Candida*-initiated changes in BM cell phenotypes following primary challenge with low virulence *C. dubliniensis* (*Cd*), together with correlates to protection. **Results:** Compared to naïve mice, we found that *Cd* induces an expansion of hematopoietic stem/progenitor cells (HSPCs) in the BM by 1 day post-inoculation (dpi), including an increase in myeloid-biased multipotent progenitor 3 (MPP3) cells at the expense of lymphoid-biased MPP4 cells (3 dpi). We also observed a rapid decrease in common myeloid progenitors (1 dpi), which occurred concurrently with a significant increase in granulocyte-macrophage progenitors and was followed by an increase in putative MDSCs (3 dpi). Additionally, *Cd*-exposed BM exhibited enhanced cytokine production compared to naïve BM when restimulated in vitro. Finally, depletion of HSPCs following *Cd* inoculation abrogated protection against lethal IAI. **Conclusion:** These results suggest that *Cd*-induced expansion/training of HSPCs and putative MDSCs in the BM is critical to protection. Future studies will mechanistically and epigenetically characterize HSPC training by *Cd* in the BM.

146B

Interactions of macrophages with white and opaque *Candida albicans*

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Abstract

Candida albicans resides asymptomatically in the gastrointestinal tract as a part of most healthy people's microbiome. In immunocompromised patients however, invasive infections can occur with mortality rates exceeding 40%. At least one third of a/a *C. albicans* strains isolated from patients have the ability to stochastically switch between two cell types, white and opaque, which are heritable for many generations. White and opaque cells differ in their metabolism, ability to mate, response to environmental cues, and interactions with the host innate immune system. We have previously demonstrated a difference in host phagocytic cell interactions with white and opaque cells, with white cells preferentially phagocytosed. To further characterize these interactions, we monitored phagocytosis of opaque cells from a library of 197 transcription factor deletion strains. We identified several transcription factors, including ones that regulate white-opaque switching, that contribute to the differential phagocytosis of the two cell types. We used time-lapse microscopy to evaluate real-time interactions between opaque cells and macrophages. In addition to decreased phagocytosis, the opaque cells that are phagocytosed proliferated within the macrophage. Furthermore, we observed decreased cytotoxicity and inflammatory responses in macrophages co-cultured with opaque cells compared to those co-cultured with white cells, providing further evidence that unlike white cells, opaque cells do not kill the macrophage in the manner as white cells. These results indicate that opaque cells induce a dampened macrophage immune response in comparison to white cells and suggests that opaque cells once phagocytosed respond very differently to that environment than do white cells.

147C

Microbiota-dependent murine immune response to intestinal *Candida albicans* colonization

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Abstract

Candida albicans is the most common fungus found in the intestinal tract of healthy individuals. Microbial dysbiosis is a common risk factor for life-threatening disseminated candidiasis; in addition, increasing evidence indicates that intestinal bacteria and fungi control *C. albicans*, and that both, as part of the microbiota, influence host immune responses. Nevertheless, the interplay between the microbiota and the immune system is still not completely understood.

The impact of the microbiota and *C. albicans* colonization on the host response was determined in genetically identical mice that differ in their fecal microbiome. Colonization of these mice with *C. albicans* for 14 days revealed similar colonization rates, which were increased after depleting the bacterial diversity by the use of antibiotics. Surprisingly, untreated mice harboring different microbiomes vary in their immune composition in Peyer´s patches but not in the spleen or in circulating immune cells. Intestinal *C. albicans* colonization displayed robust systemic B cell activation and an increase in circulating lymphocytes in all mice, while they differed in other cellular immune reactions depending on the microbiome composition. Additionally, transcriptome analysis revealed colonization induced regulation of steroid metabolic processes and organic hydroxy compound metabolic process in the distal colon, independent from the microbiota composition.

Thus, genetically identical mice harboring different microbiota show differences and similarities in their immune response to *C. albicans* colonization. Further experiments aim to understand the kinetics of the host responses and the mechanisms mediating the observed differences.

148A

Extracellular nucleotide metabolism in the context of *Candida*-neutrophils interaction

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Abstract

Host innate immunity is fundamental to the resistance against *Candida albicans* and *Candida glabrata* infection, two of the most important agents of human fungal infections. Phagocytic cells such as neutrophils, constitute the first line of host defence mechanisms, and the release of neutrophil extracellular traps (NETs) represent an important strategy to immobilize and to kill invading microorganisms, preventing the establishment of infection. The purinergic system operates an important role in immunity and inflammation homeostasis, and ectophosphatase and ectonucleotidase activities are recognized as essential for survival strategies and infectious potential of several pathogens. The expression and unique activity of a 3'-nucleotidase/nuclease (3'NT/NU), able to hydrolyse not only AMP but also nucleic acids, has been associated as part of a possible mechanism of microbes escape from NETs.

The aim of the present study was to evaluate yeasts escape to NET-mediated killing, through its 3'NT/NU enzymatic activity contribute to NET-hydrolysis.

After demonstrating *C. albicans* and *C. glabrata* 3'NT/NU activity, we show that, during neutrophils-*Candida* interaction, when NETs formation and release are triggered, NETs digestion occurs and this process of NETs disruption promoted by yeast cells was prevented by ammonium tetrathiomolybdate (TTM), a 3'NT/NU inhibitor.

In conclusion, although the exact nature and specificity of yeasts ectonucleotidases are not completely established, we highlight the importance of those enzymes in the context of infection, helping yeasts to overcome host defences, and suggesting that *C. albicans* and *C. glabrata* can escape NET-mediate killing through its 3'NT/NU activity.

149B

Candida-associated virulence and host damage modulates induction of protective trained innate immunity against fungal/bacterial sepsis

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Abstract

Background: Protection against lethal *Candida albicans* (*Ca*)/*Staphylococcus aureus* (*Sa*) intra-abdominal infection (IAI)-mediated sepsis can be afforded by prior vaccination (primary challenge) with low virulence *Candida* species (i.e. *C. dubliniensis*) that infiltrate the bone marrow (BM) and induce putative myeloid-derived suppressor cells (MDSCs) as a novel form of trained innate immunity (TII). In contrast, virulent *Candida* species, even at sub-lethal inocula, fail to protect to the level of low virulent species. The purpose of the present study was to determine how various *Ca* species with varying levels of reported virulence (via models) influence the BM *in vivo* (i.e, damage) as a measure of MDSC induction and protection. **Methods:** Mice were vaccinated (i.p.) with varying inocula of *Ca* species, including those defective in hyphal formation or candidalysin production, followed by lethal i.p. challenge (*Ca/Sa*) 14 days later and observed for sepsis (sepsis scoring) and mortality. BM was collected in a saline suspension at varying times post-vaccination to assess parameters of tissue/cell damage (hemolysis, LDH). **Results:** Vaccination with putative low virulence *C. albicans* species (i.e., ece1$^{-/-}$, 529L, TNRG1) failed to result in the same level of protection of other low virulence non-albicans *Candida* species even at sub-lethal vaccination inocula. This low level protection was concomitant with substantial evidence of damage in the BM as evidenced by increased LDH and hemolysis in BM suspensions. **Conclusion:** Vaccination induced protection by *Candida* species correlates with tissue/cellular damage in the BM as a predictive indicator of MDSC induction for protection against lethal sepsis.

150C

Impaired neutrophil extracellular trap-forming (NETosis) capacity contributes to vaginal immunopathology and susceptibility to vulvovaginal candidiasis

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Abstract

Vulvovaginal candidiasis (VVC), caused by *Candida albicans,* is characterized by hallmark robust migration of polymorphonuclear neutrophils (PMNs) into the vaginal lumen. Despite their abundance, PMNs fail to clear *C. albicans* resulting in chronic vaginal immunopathology. Failure to kill *C. albicans* is due to the presence of heparan sulfate in vaginal secretions that inhibits the interaction of PMNs with *C. albicans* hyphae. As neutrophil extracellular traps (NETs) are a major mechanism of *C. albicans* hyphal entrapment and killing, we sought to investigate PMN NETosis activity through *in vitro* and *in vivo* models simulating VVC-susceptible and -resistant conditions. Results from immunofluorescence assays revealed that formation of NETs (extracellular DNA) and release of antimicrobial proteins (histones, neutrophil elastase, myeloperoxidase) occurred in PMN-*C. albicans* cocultures using vaginal conditioned medium (VCM) generated from CD1 (VVC-resistant) mice, similar to NET-inducing positive controls. Under these NETotic conditions, PMNs showed substantial *C. albicans* killing. In contrast, PMN-*C. albicans* cocultures in VCM from C3H/HeN (VVC-susceptible) mice or culture medium containing purified heparan sulfate lacked evidence of NET formation concomitant with reduced antifungal activity. Similar results were observed *in vivo*; inoculated CD1 mice exhibited significant NET-forming PMNs interacting with *C. albicans* hyphae followed by fungal clearance within 10 days post-inoculation, whereas inoculated C3H/HeN mice sustained high vaginal fungal burden throughout the 10-day period with no evidence of NETs. Finally, expression of a putative NETosis marker, Ki67, was significantly reduced in PMNs under VVC-susceptible conditions compared to VVC-resistant conditions. These results suggest that VVC-associated immunopathology involves impaired NET-mediated antifungal activity.

151A

Syk/CARD9 signaling mediates anti-Candida protection species-specifically through hematopoietic cells in chimeric mice

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Abstract

The spleen tyrosine kinase (Syk) and the downstream adaptor protein CARD9 are crucial signaling molecules in antimicrobial immunity. *Candida parapsilosis* is an emerging fungal pathogen with high incidence in neonates while *C. albicans* is the most common agent of candidiasis. While signaling through Syk and CARD9 promotes protective host mechanisms in response to *C. albicans*, its function in immunity against *C. parapsilosis* remains unclear. Here, we generated $Syk^{-/-}$ and $CARD9^{-/-}$ bone marrow chimeric mice in order to study the role of Syk/CARD9 signaling in immune responses to *C. parapsilosis* compared to *C. albicans*. We demonstrate various functions of this pathway (e.g. phagocytosis, phagosome acidification, killing) in *Candida*-challenged bone marrow derived macrophages with differential involvement of Syk and CARD9 along with species-specific differences in cytokine production. We report that $Syk^{-/-}$ and $CARD9^{-/-}$ chimeras rapidly display high susceptibility to *C. albicans* while *C. parapsilosis* infection exacerbates over a prolonged period in these animals. Our results establish that Syk and CARD9 contribute to anti-*C. parapsilosis* immunity both *in vitro* and *in vivo*. Additionally, we confirm prior studies but also detail new insights into the fundamental roles of both proteins in immunity against *C. albicans*. Furthermore, our data suggest that Syk has a more prominent influence on anti-*Candida* immunity than CARD9. Therefore, our study reinforces the Syk/CARD9 pathway as a potential target for anti-*Candida* immune therapy.

152B

Investigation of the interaction of keratinocytes and *Candida* species

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Abstract

Our skin provides immunological protection against several pathogens. Skin epithelial cells respond to microbial stimuli in various ways, such as through the production of antimicrobial peptides or secretion of cytokines, although phagocytosis of potentially evading microbes was also reported.

Relatively little is known about how skin keratinocytes differentiate between the presence of pathogenic and commensal fungi. In this project, we aimed to investigate how human keratinocytes interact with different *Candida* species, as common colonizers of the skin. While *C. albicans* is a common cause of cutaneous candidiasis, *C. parapsilosis* is rarely associated with this disease. For the experiments human skin keratinocyte cell lines (HaCaT, HPV-KER) were applied and challenged with *C. albicans* (SC5314 and WO1 strains) and *C. parapsilosis* (GA1 and CLIB214 strains) strains. We aimed to determine the extent to which *C. albicans* and *C. parapsilosis* damage human keratinocytes, their attachment to host cells, the keratinocytes’ ability to internalize these fungi and to examine cytokine production in response to stimuli.

Our results suggest that *C. albicans* causes significantly more damage to human keratinocytes than *C. parapsilosis* and the HPV-KER cell line was more susceptible to the infection. In both HaCaT and HPV-KER cells, the production of IL-6, IL-8, and CCL5 increased primarily after *C. albicans* infection. Based on the adhesion studies, there was a low degree of association in case of *C. parapsilosis* GA1 and CLIB214 compared to *C. albicans* SC5314 and WO1.

153C

Extracellular RNA as a potential activator of Neutrophil Extracellular Traps by Candida albicans biofilm

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Abstract

Neutrophils represent the first line of innate host defense. The ability to inhibit the development of infections is associated with the involvement of several fighting strategies. The still poorly understood mechanism is netosis, involving the release of Extracellular Neutrophil Traps (NETs). NETs are complexes of chromosomal DNA and granule content. Such a web-like structure inhibits the spread of invaders.

Netosis plays a significant role in combating *Candida albicans* infections. It has been shown that several factors, composing *C. albicans* cell surface mediate NETs production. However, the development of difficult to eradicate fungal infection is associated with the formation of the biofilm structure, which partially protects the pathogen cells from contact with the host's immune system. One of the reasons for the creation of a such protective environment is the production of the extracellular matrix (ECM). The major components of the *C. albicans* ECM layer are lipids, proteins, carbohydrates but also extracellular nucleic acids, among which we observed a significant RNA content.

Considering that the ECM consisting of RNA molecules is one of the first lines of contact between biofilms and neutrophils, our current studies aimed to assess the potential role of extracellular RNA in the triggering of the netosis process by human neutrophils *in vitro*. We showed that RNA purified from *C. albicans* biofilm structure and the whole cells have the capability to induction of ROS-dependent netosis pathway. Additionally, cell migration analysis indicate that RNA molecules may also be an effective chemotactic agent. This work was supported by NCN (2019/33/B/NZ6/02284).

154A

BIOFILM FORMATION AND ADHESION CAPABILITY OF Candida spp.CLINICAL ISOLATES

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Abstract

Background:

Yeasts of genus Candida are among ten most common pathogens in hospitals surgical profile accounting for 17% of the total number of infectious complications in the intensive therapy units (ITU).

Objective:

The aim of this work was to evaluate the pathogenic potential of Candida isolates from patients' urine, sputum, blood and throat in the cases of nosocomial ITU infections by assessing yeasts ability for adhesion and biofilm formation (BF).

Methods:

Adhesive properties of isolated strains of microorganisms were studied on human erythrocytes of Rh-positive blood group 0(I) by Brilisa. BF capability was carried out according to Romanova.

Results.

The strains able for adhesion and therefore with higher pathogenic potential dominated. There were 30% of highly-adhesive strains and 33% strains with medium adhesion.

Highly- and medium-adhesive strains were detected in urine and blood, whereas low- and no adhesive strains were isolated from throat and sputum smears.

The highest BF ability (0.55 ± 0.12 U OD) was shown by the isolates from urine, and the lowest ability by yeast isolated from throat (0.32 ± 0.03 U OD).

None-adhesive strains were detected only for C. albicans and C. glabrata, and low-adhesive only for C. albicans. C. kruzei, C. tropicalis, C. sake, C. lusitaniae, C. parapsiolis demonstrated high- and medium-adhesiveness with the maximum values for C. krusei, C. sake species. The ability of C. non-albicans isolates for high and medium adhesion was 3 times higher than for C. albicans suggesting C. non-albicans yeasts are becoming more aggressive as nosocomial ITU infections agents.

155B

Recovery of *Candida* spp. from a denture rat model

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Abstract

Denture stomatitis is a common infection in denture wearers. This study evaluated the recovery of *Candida* spp. from the palate of Wistar rats after using an acrylic device with single and mixed-species of *Candida* spp. After approval of the Ethics Committee, 84 male and female Wistar rats were used. Custom-made acrylic devices were fabricated for each animal and sterilized by microwave irradiation. Single and mixed species biofilms of *C. albicans* (Ca), *C. glabrata* (Cg), and *C. tropicalis* (Ct) were grown on the devices for 48 h at 37 °C. Rats were anesthetized and the devices were cemented on the molar teeth (n=5 for each sex and *Candida* spp.). Rats received a carbohydrate-rich diet. Single and mixed species were inoculated in the oral cavity thrice after three-day intervals. Controls received only dentures without *Candida* spp. After 4 weeks, the devices were removed, the palates were swabbed, and diluted samples were plated on Agar Sabouraud Dextrose and CHROMAgar *Candida* for colony counting and presumptive identification, respectively, after 48 h. Data were analyzed by 3way ANOVA (α=5%). There was a significant interaction (p=0.003) between sex and species. For females, all groups recovered significant values (p≤0.027) compared with controls. For males, groups with Ct as single and dual-species showed the lowest values without difference (p≥0.183) with the control. The groups with triple-species showed the highest values but without difference (p≥0.071) with the groups with single and dual-species, except males with Ct. Ct alone showed reduced recovery from palate of male rats.

156C

Candida albicans genes expressed during peritonitis and in intra-abdominal abscesses (IAA) contribute to pathogenesis of intra-abdominal candidiasis (IAC) but not hematogenously disseminated candidiasis (DC).

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Abstract

Background. Pathogenesis of DC is much better studied than that of IAC. We hypothesized that many *C. albicans* genes that contribute to IAC pathogenesis do not contribute to DC.

Methods. We measured *C. albicans* SC5314 gene expression (RNA-Seq, in triplicate) during early peritonitis (30mins), late peritonitis (24hrs) and IAA (48hrs) of mice. Differential expression was defined by ≥2-fold differences (false discovery rate ≤0.01).

Results. ≥7 million *C. albicans* reads were detected in each experiment. 67% of *C. albicans* reads mapped to coding sequences, covering 93% of open reading frames. The 50 genes most highly expressed during early peritonitis were associated with pH (e.g., RIM101, PHR1), oxidative stress responses, and adhesion/hyphal growth (e.g., ALS3, HWP1, ECM331, SAP6). The corresponding 50 late peritonitis genes were associated with neutrophil/macrophage responses and nutrient acquisition (glyoxylate cycle, fatty acid β-oxidation, iron homeostasis). Responses within IAA included DNA damage and iron metabolism. The top 50 core gene responses for all stages were associated with adhesion, stress response, and glucose transport. Null mutants for genes involved in adhesion (ALS1, ALS3), transport (OPT8, SGE11), biofilm (ZCF23), and DNA (RFX1, RFX2, DDI1) and cell wall (DAP2) damage responses were attenuated for virulence in temporal-spatial fashion during peritonitis and/or IAA, but not during DC. Mutants for genes involved in copper metabolism were attenuated during both IAC and DC (CCC2) or DC alone (MAC1).

Conclusions. *C. albicans* encounters different environmental conditions during IAC and DC. Many *C. albicans* genes induced during IAC contribute to peritonitis and/or IAA, but not to DC.

157A

Transient or Persistent? The fate of fungi in the gut microbiota of healthy and dysbiotic individuals as recreated in a complete mucosal ileum-colon in vitro simulation using the SHIME® platform.

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Abstract

The European FunHoMic project aims at defining and exploiting the Fungal-Host-Microbiota interplay to identify novel biomarkers (fungal or host genetic polymorphisms, microbiota profiles, metabolites or immune markers) for the stratification of a patient's risk of serious fungal infection. *(A separate abstract for the whole consortium has been submitted)*

Within the framework of the project, there is a need for a representative *in vitro* model to study the interplay between fungi and bacteria in a human gut-like environment. More specifically, having a stable *C. albicans*-infection model will allow further understanding the role of dysbiosis in the pathogenicity of this commensal species of the gut; as to testing specific treatments for preventing or curing *Candida* infections. For this purpose, the SHIME® (Simulator of the Human Intestinal Microbial Ecosystem), represents a valid dynamic model of the complete gastrointestinal tract. It has already been fully validated to simulate the bacterial part of the microbiota with differences both longitudinally (ileum, ascending, transverse and descending colon) and laterally (mucus versus lumen).

Yet, prior to develop a *C. albicans*-long term infection model, there is a need to better identify the gut mycobiota niche. This is done by quantifying the fungal load (qPCR) from each gut compartment and from different donors (healthy or disease status), but also by spiking five common human gut yeasts and characterizing their behavior (colonization preferences, metabolic activity) *(batch experiment first).*

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 812969.

158B

In vivo analysis of hyphal morphogenesis in *C. albicans*.

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Abstract

The morphogenetic switch between yeast and hyphal forms of the human fungal pathogen, *Candida albicans*, is critical to invade and thereby establish virulence in the host. *In vitro*, a core set of transcription factors (TFs) govern the dimorphic switch. However, the transcriptional requirements for filamentation vary with the specific environments raising the possibility that distinct TFs network may mediate filamentation in the mammalian host. To address this question, we have initiated the first systematic large-scale screen approach to characterize the TFs network that orchestrates filamentation *in vivo*. To do this, the dermal layer of the DBA/2N mouse ear was inoculated with fluorescently labelled *C. albicans* and the morphology of reference vs TF mutants was assessed after 24 hours in the ears of living mice using confocal microscopy. To date, we have screened 70 TF mutants with morphology defects *in vitro*. Our *in vivo* data identified a TF *FGR15* and *CPH2* that formed filamentation *in vitro* whereas they were compromised to do so *in vivo.* We also identified three TFs *AAF1, ISW2,* and *HMS1* that under *in vitro* conditions were defective in filament formation but *in vivo* formed a strong filamentation. In support to our hypothesis, we have identified a new set of TFs that are specifically regulates *in vivo* morphogenesis. Ongoing studies will focus on identifying the mechanistic basis that contributes to and governs the pathogenicity in *C. albicans* within the host.

159C

Interactions during Polymicrobial Infection Affect Anti-fungal Treatment Success

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Abstract

Candida albicans is an opportunistic fungus that can cause invasive candidiasis with 40 % mortality in hospitals. *Pseudomonas aeruginosa* is an important pathogen responsible for respiratory tract infections in cystic fibrosis (CF) patients. *C. albicans* and *P. aeruginosa* can be both found in the lungs of CF and mechanically ventilated patients. Polymicrobial infections can be challenging to treat mainly because of lack of understanding of different interactions taking place during co-infections, but also the efficacy of drugs used to treat the co-infection. Furthermore, environmental cues have been shown to affect the interactions between microorganisms during polymicrobial infection and therefore the effectiveness of the treatment administered. In our work, we investigate the efficacy of anti-fungal treatment during *C. albicans*-*P. aeruginosa* co-infection. We are performing *in vitro* and *in vivo* experiments using the zebrafish swimbladder infection model. Zebrafish are a powerful tool that provide a simple environment but also allow us to study complex interactions between host and pathogens. Interestingly, we found that fluconazole (Flc) treatment, an antifungal drug that only stops the growth of *C. albicans*, is more effective at killing *C. albicans* during co-infection. Flc becomes fungicidal causing death of *C. albicans* during co-infection, both *in vitro* and in the swimbladder infection. We also found that addition of iron partially prevents *C. albicans* death both *in vitro* and during infection, suggesting that micronutrient availability dictates the success of antifungal drugs. Our results suggest that iron availability plays an important role during *Pseudomonas*-*Candida* coinfection and highlight how treatment effectiveness can be environment-dependent.

161B

CO2 accelerates *Candida albicans* biofilm formation and reveals novel approaches to their inhibition on airway management devices

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Abstract

C. albicans is the predominant human fungal pathogen worldwide and frequently colonises medical devices, such as voice prosthesis, as a biofilm. It is a dimorphic yeast that can switch between yeast and hyphal forms in response to environmental cues, a property that is essential during biofilm establishment and maturation. One such cue is elevation of CO_2 levels, as observed in exhaled breath for example. However, despite the clear medical relevance the effects of high CO_2 levels on *C. albicans* biofilm growth has not been investigated to date. Here, we show that 5% CO_2 significantly enhances each stage of the *C. albicans* biofilm forming process; from attachment through maturation to dispersion, via stimulation of the Ras/cAMP/PKA signalling pathway. Transcriptome analysis of biofilm formation under elevated CO_2 conditions revealed the activation of key biofilm formation pathways governed by the central biofilm regulators Efg1, Brg1, Bcr1 and Ndt80. Biofilms grown in under elevated CO_2 conditions also exhibit increases in azole resistance, tolerance to nutritional immunity and enhanced glucose uptake capabilities. We thus characterise the mechanisms by which elevated CO_2 promote *C. albicans* biofilm formation. We also investigate the possibility of re-purposing drugs that can target the CO_2 activated metabolic enhancements observed in *C. albicans* biofilms. Using this approach we can significantly reduce multi-species biofilm formation in a high CO_2 environment and demonstrate a significant extension of the lifespan of voice prostheses in a patient trial. Our research demonstrates a bench to bedside approach to tackle Candida albicans biofilm formation.

162C

Finding new genetic determinants of Candida albicans through microevolution in the oral mucosa

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Abstract

The high genetic diversity within the fungal species *Candida albicans* results in different phenotypic landscapes, which determine the outcome of fungal-host interactions. However, identifying the causative genetic factors that underlie a specific phenotype remains a significant challenge. To maximise the chances of pinpointing novel genetic factors responsible for functional changes within the species of *C. albicans*, we applied a within-host microevolution approach. We predicted that this would generate closely related strains of *C. albicans* that vary in their phenotype but remain genetically similar. The highly virulent strain SC5314 only transiently colonises immunocompetent mice in an experimental setting when infected via the oropharyngeal route. After serial infections with strain SC5314 that was previously treated with an alkylating agent, we observed prolonged persistence of the evolved strains within the murine oral mucosa. This was accompanied by a reduced induction of the inflammatory antifungal response in the host, including decreased neutrophil infiltration and cytokines expression. Moreover, evolved strains exhibited a diminished capacity to trigger cellular damage in epithelial cells *ex vivo*. All these phenotypic attributes are characteristic of low-virulent strains of *C. albicans*. Therefore, our findings indicate that *C. albicans* adapts to the host by adopting commensal-like features in the oral mucosa of immunocompetent mice without antibiotic treatment.

163A

Device-associated *Candida* biofilms inhibit the function of neutrophils isolated from candidiasis patients

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Abstract

Background: On indwelling catheters, *C. albicans* forms biofilms that resist clearance by host defenses. Neutrophils are leukocytes essential for eradicating *Candida*. The antifungal activities of neutrophils from healthy donors are impaired by *C. albicans* biofilms. However, how neutrophils respond to *C. albicans* biofilms in the setting of invasive candidiasis is unknown.

Methods: Patients with invasive candidiasis (determined by the presence of *Candida* at a normally sterile site) and healthy participants were enrolled through IRB-approved protocols. We isolated peripheral blood neutrophils and analyzed their interactions with *C. albicans* during biofilm and planktonic growth. We analyzed fungal killing (PrestoBlue assay), neutrophil extracellular trap formation (NET) formation (Sytox green, scanning electron microscopy, immunofluorescence labeling of citrullination) and ROS production (CM-H2DCFDA fluorescence).

Results: When compared to healthy participants, neutrophils collected from patients with invasive candidiasis exhibited elevated basal activation and amplified NET and ROS responses to a potent stimulus (PMA). In response to planktonic *Candida*, the neutrophils from patients generated ROS and formed NETs to a level similar to that observed for healthy participant neutrophils. Upon encounter with biofilms, both patient and healthy participant neutrophils did not significantly produce ROS or NETs beyond their basal level. Neutrophils from patients were more active against planktonic *Candida* than biofilm.

Conclusion: During invasive candidiasis, neutrophils display a heightened capacity for ROS and NET production. Despite increased neutrophil activation, *C. albicans* biofilms impair these processes and resist killing by neutrophils. These findings shed light on the resilience of device-associated biofilms in patients with candidemia and invasive candidiasis.

164B

Examination of *Candida albicans* morphology during commensal growth in different sections of the murine gastrointestinal tract

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Abstract

Background: *Candida albicans* is a frequent component of the human gastrointestinal (GI) microbiome. In addition to being a gut commensal, the fungus can also turn into a fatal pathogen. In order to better understand the commensal phenotype, the colonization ability and distribution of different morphological forms of this fungus were compared in conventional and germ-free mice.

Methods: C57B6 conventional (supplemented with antibiotics) and germ-free mice were colonized with SC5314. Fungal load was determined from CFU (colony forming unit) analysis on tissues obtained from different parts of the GI. The two different morphological forms, yeast and hyphae, were enumerated after staining paraffin sections with anti-*Candida* antibody. Furthermore, the distribution of different cell types across the GI was imaged using fluorescence in situ hybridization (FISH).

Results: Comparable CFUs were obtained from germ-free and conventional mice. A gradual increase was observed in CFU counts from duodenum to colon in the case of both conventional and germ-free mice. We also observed a relative decrease in the proportion of yeast cells and an increasing fraction of hyphae from duodenum to colon. FISH suggests that yeast cells are generally located closer to the epithelium while hyphae tend to colonize more in the lumen.

Conclusion: Highest levels of fungal colonization were observed in the ileum and colon of both conventional and germ-free mice, and these organs also consistently contained a higher proportion of hyphal cells than yeast cells. These results highlight the different micro-environments encountered by *C. albicans* in the GI tract, and how these can influence the yeast-hyphal dichotomy.

165C

Clinical *Candida albicans* Isolates have Three Different Virulence Phenotypes

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Abstract

Introduction. Although clinical isolates of *Candida albicans* differ in virulence in mouse models of infection, the mechanisms for this variability are incompletely understood. Using different strains of *C. albicans*, we set out to analyze strain-dependent differences in host cell interactions and virulence during oropharyngeal candidiasis (OPC) and hematogenously disseminated candidiasis (HDC).
Methods. The capacity of 15 *C. albicans* isolates to damage to oral epithelial cells and vascular endothelial cells *in vitro* was determined. The virulence of six these isolates was assessed in immunocompetent mouse models of OPC and HDC. **Results.** Three distinct phenotypes were identified. Two strains (529L and CEC3681) caused low damage to both epithelial and endothelial cells. In mice, these strains had a commensal phenotype, persisting in the oral cavity during OPC and causing delayed mortality during HDC. Five strains (including SC5314 and CEC3605) caused high damage to both cell types. In mice, strains SC5314 and CEC3605 had a virulent phenotype, failing to persist in the oropharynx during OPC and causing rapid mortality during HDC. The remaining 8 strains (including CA101 and CEC3672) caused low damage to epithelial cells, but high damage to endothelial cells. In mice, strains CA101 and CEC3672 had a flexible phenotype, persisting in the oropharynx during OPC, but causing rapid mortality during HDC. **Conclusions.** Different *C. albicans* strains have three different host cell damage phenotypes *in vitro,* which correlate with their virulence during OPC and HDC. Transcriptional profiling experiments are currently underway to delineate the molecular basis for these three distinct phenotypes.

166A

Modulation of *C. glabrata* response and resistance to azoles in the presence of acetic and lactic acids at low pH

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Abstract

Vulvovaginal candidiasis is the leading superficial infection caused by *Candida*, with *C. glabrata* being among the more relevant causative species. Azoles are typically used for treatment of these infections, however, the success of this approach is limited in *C. glabrata* due to its high resilience to these drugs and genomic plasticity that renders it capable of rapidly acquiring resistance. A comprehensive knowledge on the players mediating response and tolerance to azoles in *C. glabrata* has been obtained, however, this was mostly performed under neutral conditions while in the vaginal tract the pH is acidic(~4.5) due to the accumulation of acetic and/or lactic acids produced by the commensal bacteria. In this work the effect in azole resistance of acidic pH, either using a strong acid or acetic/lactic acids as acidulants, was investigated. *C. glabrata* cells were found to be much more resilient to fluconazole and miconazole at acidic pH (both when a strong acid or acetic/lactic acids are used as acidulants) than under neutral conditions and this correlated with a higher accumulation of the drugs in the latter conditions. The reasons underlying this phenotype will be discussed with emphasis on the role played by the structure of the cellular envelope and the activity of presumed azole efflux pumps. Results from phenotyping of an available collection of deletion strains for susceptibility to fluconazole under acidic conditions (using a strong acid or acetic/lactic acids as acidulants) will also be discussed, with emphasis on the role of newly identified players.

168C

FunHoMic: A Marie Skłodowska-Curie Innovative Training Network for the study of the Fungus-Host-Microbiota interplay

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Abstract

Introduction

The FunHoMic project is a Marie Skłodowska-Curie Innovative Training Network comprising 13 PhD students, 8 academic partners and 3 industry partners aiming to understand the interplay between fungi, hosts and microbiota to improve prevention and treatment of fungal infections.

Importance

About 2 billion people suffer fungal infections, which have a mortality rate close to that of malaria or breast cancer. *Candida albicans* has a high clinical and economic burden, making it of particular interest to the FunHoMic project. 70% of women experience at least one episode of vulvovaginal candidiasis (“thrush”) during their lifetime; 8% suffer recurring infections. *C. albicans* may live as a commensal but can cause symptoms when the fungus-host-microbiota equilibrium is disrupted. Infections by *C. albicans* have a significant clinical impact, with fatalities in severe cases. Many factors are associated with *C. albicans* infections; intensive care, neutropenic and diabetic patients are most at risk of systemic infection. Rising antifungal drug resistance has led to certain *C. albicans* infections having no treatment option.

Aim

The FunHoMic consortium combines projects on fungal pathogenesis, immunology, microbial ecology and ’omics technologies to understand and exploit interactions between fungus, host and microbiota. Identification of novel biomarkers on the fungal side such as genetic polymorphisms or on the host side such as microbiota profiles, metabolites and/or immune markers can lead to patient classification based on relative risk of infection. This could be the beginning of personalised management for fungal infections using preventive or therapeutic interventions like new antifungals, immune modulators or Live Biotherapeutic Products (LBPs).

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 812969.

169A

The Microbial Ecology of *Candida albicans* Strains CHN1 and SC5314 in Mice

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Abstract

Strain SC5314 is the most widely studied strain of *Candida albicans*. Despite *C. albicans* being the most commonly isolated yeast from the human gastrointestinal (GI) microbiome, strain SC5314 does not stably colonize the mouse GI tract long term, even after antibiotic disruption. In contrast, strain CHN1 will stably colonize the mouse GI tract long term. Comparative genomic analysis of strain CHN1 indicates that it belongs to a different evolutionary clade of *C. albicans* than strain SC5314. Previous studies from our laboratory have shown that colonization by strain CHN1 causes a change in the GI bacterial microbiome of mice and predisposes them to more robust Th2 immune responses. Despite this, little is known about the GI microbial ecology of SC5314 vs. CHN1 and subsequent host responses. Using a short-term antibiotic disruption model in C57BL/6 mice, we have been able to observe significantly different colonization kinetics between these two *C. albicans* strains, with CHN1 establishing stable long-term colonization. In contrast, colonization by SC5314 was lower, highly variable and cage-dependent. *C. albicans* colonization kinetics impacted the composition of the bacterial microbiome with a marked effect on the levels of *Lactobacillus* and *Enterococcus*. qPCR analysis of 46 host immune response genes did not detect significant differences in host gene expression between SC5134 and CHN1 colonized mice, except for chitinase expression. Thus, these studies suggest that yeast-bacteria interactions in the microbiome may be far more important in determining long-term colonization potential of *C. albicans* and secondary immunomodulatory effects.

170B

Global Analysis of Circuitry Governing *Candida albicans* Morphogenesis within Host Immune Cells

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Abstract

The evasion of killing by host immune cells is crucial for fungal survival in the host. For the human fungal pathogen *Candida albicans*, morphogenesis upon internalization by macrophages is a key intracellular survival strategy that occurs through mechanisms which remain largely enigmatic. To identify the *C. albicans* genes that orchestrate filamentation in the macrophage, we performed a functional genomic screen of conditional expression mutants covering ~40% of the genome and identified 298 genes important for filamentation upon phagocytosis. Notably, fifty-four of the genes were dispensable for filamentation in response to serum, demonstrating specificity in the program governing morphogenesis within macrophages. To discover circuitry through which one of these genes enables filamentation, we performed selection experiments to restore filamentation in a strain lacking *MSN5*, which encodes a predicted karyopherin for the nuclear import and export of proteins. Whole genome sequencing of evolved lineages uncovered potential filamentation-restoring genetic alterations. Further, we explored filamentation-inducing stimuli within the macrophage and determined that macrophage lysate is sufficient to induce morphogenesis. Bioactivity-guided fractionation coupled to mass spectrometry identified the immune modulator, prothymosin alpha (PTMA), as a potential macrophage-derived trigger of filamentation. Immunoneutralization of PTMA from macrophage lysate abolished its ability to stimulate *C. albicans* filamentation, supporting PTMA as a filamentation-inducing component of the lysate. This work is the first to implicate a specific host protein as a trigger of filamentation and identifies key elements of the regulatory circuitry uniquely governing *C. albicans* morphogenesis in response to phagocytosis by host immune cells.

171C

Preliminary study into the effects of tobacco smoke on *Candida albicans.*

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Abstract

Background: Denture-stomatitis (DS) is the most common form of oral candidosis with increased prevalence in cigarette smokers (Akram *et al.* 2018). Interestingly, tobacco condensate (TC) increases *Candida albicans* adhesion, growth, biofilm-formation, virulence gene expression (Semlali *et al.* 2014) and hyphal production (Awad and Karuppayil 2018). We hypothesised that TC-treated denture acrylic would therefore affect *C. albicans* within acrylic biofilms.

Methods: Acrylic discs (pre-conditioned with TC, artificial saliva (AS) or water) were incubated at 37°C with *C. albicans* (n=6) for 90 min or 24 h. Adherent *Candida* were stained with calcofluor white and confocal laser scanning microscopy (CLSM) used to assess levels of adherence, biofilm and hyphal numbers. Expressed virulence genes (n=7) were measured by qPCR.

Results: CLSM showed that effects of TC-treatment were strain dependent. Adherence of *C. albicans* PTR/94 to TC-treated surfaces was significantly ($P<0.002$) lower than on the untreated control. Biofilm levels of PTR/94 after 24 h were found to be significantly higher on AS-treated acrylic than the TC-treated and untreated control. Five strains had significantly fewer filamentous forms after 90 min on TC-treated surfaces. TC-treatment promoted hyphal levels for strain 705/93 after 24h.

Conclusion: TC pre-conditioning altered adherence and biofilm coverage of *C. albicans* to acrylic surfaces and influenced hyphal development. Work is ongoing to ascertain the significance of these effects on *C. albicans* pathogenicity.

Akram *et al.* (2018). *Journal of Oral Science* **60(1):**115–120.

Awad and Karuppayil (2018). *American Journal of Clinical Microbiology and Antimicrobials* 1**(3):**1–6.

Semlali *et al.* (2014). *BMC Microbiology*. **14:**61

172A

Candida albicans morphological switch and microbiota changes in the elderly gut: impact of adenosine A_{2A} receptors

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Abstract

The observed lifespan in modern human societies is not a synonym of healthspan, with the reduction of the immune and metabolic systems effectiveness, critical keys in the settlement of high disability and morbidity rates and loss of quality of life. Also, ageing is a critical factor contributing to the observed increase in the pathogenicity of microbes usually considered harmful. The adenosine A_{2A} receptor ($A_{2A}R$) contributes to fine-tuning inflammatory and immune responses, prompting an efficient elimination of threats while minimizing tissue damage.[3]

In an *in vivo* mice model of aged, adult and young individuals we assessed the relative intestinal over-colonization by *Candida albicans*, and the correlation between this and the gut $A_{2A}R$ distribution/density, tissue damage; wild type and $A_{2A}R$ knockout mice gut microbiota diversity was also studied. We showed that elderly mice are more prone to over-colonization by *C. albicans.* This is related with higher growth rate in the intestinal lumen, which is independent of gut tissues invasion but with higher inflammation. These mice have a higher stomach colonization and increased yeast-to-hypha transition, when compared with young and adults. Also, aged mice have lower gut $A_{2A}R$ density and *C. albicans* overgrowth failed to increase it, as observed in younger mice. The lack of $A_{2A}R$ leads to changes in the microbiota, including an increase of Enterobacteriales and a decrease of Clostridiales elements.

These results indicate that aged mice have lower ability to cope with inflammation due to *C. albicans* over-colonization and morphological switch, related with the inability to adaptively adjust $A_{2A}R$ density.

173B

The Tog1 pathway: in the crossroads of oxidative stress resistance and virulence in *C. glabrata*.

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Abstract

Understanding the molecular basis of the unique ability exhibited by *Candida glabrata* to survive the harsh environment of host immune cells is crucial to deal with this emerging pathogen.

In this presentation, the transcription factor CgTog1 is presented as a new determinant of *C. glabrata* virulence. CgTog1 was found to be required for oxidative stress resistance and for the modulation of reactive oxygen species inside *C. glabrata* cells. As revealed through RNA-seq-based transcriptomics analysis, CgTog1 was found to up-regulate the expression of 147 genes in *C. glabrata* cells exposed to H_2O_2. Given the importance of oxidative stress response in the resistance to host immune cells, the effect of *CgTOG1* expression in yeast survival upon phagocytosis by *Galleria mellonella* hemocytes was evaluated, leading to the identification of CgTog1 as a determinant of yeast survival upon phagocytosis. CgTog1-mediated survival upon phagocytosis is probably correlated with its targets, comprising many genes whose expression changes in *C. glabrata* cells after engulfment by macrophages, including those involved in reprogrammed carbon metabolism, glyoxylate cycle and fatty acid degradation.

In summary, CgTog1 is a new and specific regulator of virulence in *C. glabrata*, contributing to oxidative stress resistance and survival upon phagocytosis by host immune cells.

174C

The gut commensal *Bacteroides vulgatus* mpk reduces *Candida albicans* pathogenicity towards epithelial cells

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Abstract

The human body is colonized by various microbes, among them the yeast *Candida albicans*. Mostly harmless, this opportunist causes also disease, ranging from superficial infections to sepsis. Risk factors are disturbed host defenses, mucosal barrier breakdown, and antibiotic-induced dysbiosis. Hence, residing bacteria are important to protect from *Candida*-mediated damage or inflammation. *Bacteroides vulgatus* mpk, e.g., is described as positively immunomodulatory in mouse models of inflammatory bowel disease, but its effect on the mycobiota is unknown.

In this study we aimed to determine if *B. vulgatus* mpk affects *C. albicans* pathogenicity.

Therefore, intestinal and oral epithelial cells were pre-infected *in vitro* with *B. vulgatus* mpk and then challenged with *C. albicans* SC5314. The role of soluble factors was investigated by spatial separation or use of *Bacteroides*-conditioned medium (BCM).

Preincubation of host cells with *B. vulgatus* mpk strongly reduced *C. albicans*-mediated damage while fungal burden and hyphal length were unaffected by the bacterium. The protective effect did not depend on direct contact of *Bacteroides* to host cells or *Candida* and could be mimicked using BCM. Contact independency suggests that diffusible factors modulate host cell susceptibility.

Ongoing experiments aim to identify key soluble *Bacteroides* mediators as well as subsequent host cell signaling. Additionally, co-colonization experiments of germ-free mice are planned to investigate *B. vulgatus* mpk's potential to mediate colonization resistance towards *C. albicans*. This will contribute to our understanding of how commensal bacteria affect *C. albicans* and host protection.

175A

Albumin reduces *Candida albicans*-induced damage through neutralization of candidalysin

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Abstract

Albumin is abundant in serum but is also excreted at mucosal surfaces and enters tissues when inflammation increases vascular permeability. Host-associated opportunistic pathogens such as *Candida albicans* encounter albumin during commensalism and when causing infections. We, therefore, investigated the role of albumin on the pathogenicity of *C. albicans*.

We found that albumin inhibited *C. albicans*-induced damage on various host cell types. Mechanistically, we demonstrated that the reduced damage was due to an efficient neutralization of the toxin candidalysin by albumin. Our analysis revealed that the basis for the neutralization of candidalysin is binding through hydrophobic interactions with albumin. We discovered that albumin can similarly neutralize a variety of fungal (α-amanitin), bacterial (Streptolysin O, Staurosporin), and insect (melittin) hydrophobic toxins. Collectively, these results provide a fundamentally important defence mechanism against toxins and a potential key role for albumin in pathogenesis of *C. albicans* infections.

176B

Candida albicans and the host: the secrets of adaptation

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Abstract

The commensal fungus *Candida albicans* displays a high intraspecies diversity. Genetic variations result in phenotypic and functional differences between strains, which affect the fungus-host interaction. It remains currently unclear how individual strains of *C. albican*s adapt to the host environment to which they are exposed during commensalism, and how this adaptation might contribute to diversification of the species. To tackle this question, we orally colonized mice with the low-virulent strain 101, which persists in the murine oral mucosa like a commensal, and assessed its adaptation over time. Analysis of re-isolated fungus revealed rapid, albeit reversible, phenotypic changes, such as accelerated growth and enhanced filamentation. The adaption process is conserved across different strains of *C. albicans* and in immunologically distinct hosts. In immunodeficient mice, however, which lack the IL-17 pathway (which is essential for maintaining homeostatic fungal colonization), *C. albicans* adopts a more invasive and pathogenic phenotype leading to disruption of tissue homeostasis. The molecular basis underlying these changes both on the fungal and host side are currently under investigation.

177C

C. albicans strains SC5314 and 101 have similar potential to colonize the murine gut and colonization protects from subsequent systemic infection

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Abstract

Candida albicans can colonize various mucosal surfaces including the gut and the oral cavity. In the latter, colonization efficacy depends on strain-specific characteristic and the induction of host responses: While *C. albicans* SC5314 is cleared from the oral mucosa of immunocompetent mice, strain 101 can persist for a prolonged period of time.

To determine whether these strain also differ in their ability to colonize the murine gut, female C57Bl/6 mice with and without antibiotic treatment received a single dose of either strain by oral gavage and fungal colonization was monitored by plating of fecal samples over a course of 14 days. For both strains, similar colonization levels were observed, which were higher in the presence of antibiotics.

Colonization with *C. albicans* SC5314 has been shown by the Way lab to induce systemic Th17 responses that mediate protection from subsequent systemic infection. We found that colonization with *C. albicans* 101 likewise induced systemic Th17 responses, and thus compared the susceptibility of mice colonized with either strain to systemic infection with *C. albicans* SC5314. Both strains conferred comparable levels of protection to systemic candidiasis.

The result that two *C. albicans* strains that differ significantly in their ability to colonize the oral mucosa show a more uniform behavior in gut colonization and subsequent induction of systemic protective responses suggests that strain-specificity of interactions of *C. albicans* with the host might be restricted to specific anatomical sites.

179B

The nutrient composition of the environment rather than morphology or genotype shapes the metabolome of *Candida albicans*

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Abstract

Filamentation is considered the key virulence attribute in the fungal pathogen *Candida albicans*, since it is closely tied to infection-associated processes such as tissue invasion and escape from phagocytes. Although the molecular response to several hyphal inducers has been described, it remains unclear whether hyphae-specific metabolic signatures exist.

Here, for the first time, we integrated global transcriptome and metabolome analyses for several hyphal inducing (human serum, N-Acetylglucosamine and alkaline pH) and yeast-yielding conditions to identify the core responses, central hubs and unique metabolic adaptions that accompany the filamentation process. We also included the hyphal-defective strains *hgc1*Δ (impaired in hyphal maintenance) and *cph1*Δ/*efg1*Δ (yeast-locked) to further define the specific metabolic profiles of yeast and hyphae. We aimed to find unique compounds that might be used to discriminate between those two morphotypes.

Confirming previous findings, the transcriptional response was diverse and dependent on the medium, filamentation properties of the strain, and time point. However, surprisingly, these major transcriptional changes were not reflected in the cellular metabolome, where sample clustering was primarily defined by medium composition and partially by time point. Thus, *C. albicans* undergoes morphotype-independent metabolic remodeling to adapt to the available nutrients. These results underline the importance of niche-specific nutrient composition in the host for fungal commensalism and pathogenicity. However, in this context, the presence of accompanying microbiota or certain host factors might also have an impact on the fungal metabolome and could selectively influence yeast or hyphae profiles.

180C

Integrative functional analysis uncovers metabolism of invasive Candida species

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Abstract

Background

Candida species represent the majority of fungi in the human oral mycobiome but are also the most common human fungal pathogens. Primary metabolism in these species shows high levels of plasticity to boost their ability to withstand the environmental stresses they are exposed to. The ability of *Candida* species to diversify their metabolism in response to stress is key in enabling the switch to pathogenic growth. The aim of this study is to elucidate the core differences in secondary metabolic pathway activity of clinically relevant *Candida* species.

Methods

Extracellular metabolites released from *C. albicans, C. dubliniensis, C. tropicalis, C. auris, C. krusei, C. parasilopsis* and *C. glabrata* grown in *in vivo* cultures were determined by metabolomics performed using the MaxQuant500 kit. The data were subsequently analysed using bioinformatic tools.

Results and Conclusions

There were clear differences in production of metabolites from the different *Candida* species. Moreover, nutrient requirements of biosynthetic pathways for growth and pathogenesis differed between species. *C. albicans,* in particular, showed distinct deviations, particularly within amino acid analytes. Clustering of the species identified unique clusters for *C. albicans* and *C. auris* that were distinct from other *Candida* species. Further, *C. auris* demonstrated differences in all aspects of metabolism. These findings imply that evolutionary changes in the different species are reflected in their ability to colonise the host environment. Thus, this study highlights the need to investigate the metabolic pathways of *Candida* species further to discern their impact on the host and its associated microbiota.

181A

Metabolic response to Ferulic acids in *Candida albicans* during yeast to hyphae morphogenesis captured by Fourier transform mid-infrared spectroscopy

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Abstract

Molecules derived from natural sources are promising candidates for novel therapeutic options against *Candida* infections. Such options can address clinical challenges including the need for antifungal agents and therapies to combat emerging resistant strains. Phenolic acids have shown activity against *Candida* but the mechanism of actions is largely unknown. For this purpose, Fourier transform mid-infrared spectroscopy (FT-mIR) was applied to determine the effect of Ferulic acid on *Candida albicans*' virulent attribute, yeast-to-hyphae morphogenesis. *C. albicans* SC5314 treated with and without Ferulic acid at 7 mM (pre-determined minimum concentration to inhibit 50% hyphal formation) were induced to form hyphae by a nutrient limiting media containing N-acetylglucosamine (GlcNAc) at 37 °C. Whole cell samples were collected at four time points, during a 24 h period, representing stages of hyphal formation for FT-mIR measurement. Hierarchical clustering of the FT-mIR spectra classified *C. albicans'* metabolic profiles into three groups that aligned with percentage hyphae present in the culture. However, two color coded heatmaps, generated based on the calculation of IR absorption band position shifts and intensity changes via second derivative and curve fitting, demonstrated that the structural and compositional modifications occurred even when visual morphology remained unchanged. Ferulic acid induced metabolic response of *C. albicans* independent to hyphal induction effect of GlcNAc in the spectral regions of fatty acid, amides, carbohydrates, mixed zone and nucleic acids. This work provides valuable spectral references and functional information on how phenolic acids are imbued with antifungal properties, which has diagnostic implications.

182B

A systemic analysis of amino acid transporters identifies Gnp2 as the main proline permease in *Candida albicans*

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Abstract

The tight association of *Candida albicans* with the human host has driven the evolution of mechanisms that permit metabolic flexibility. Amino acids, present in free form or peptide bound, are an abundant carbon and nitrogen source in many host niches. Further, the capacity to sense and utilize certain amino acids, like proline, is directly linked to virulence. The *C. albicans* genome encodes for at least 24 amino acid permeases (AAPs), highlighting the importance of flexible amino acid uptake for fungal growth and virulence. Although the substrate specificity and role of certain AAPs has been investigated, a comprehensive characterization was missing. Therefore, we assembled a library of AAP deletion strains, which was tested for resistance to toxic amino acid analogs. Most striking was the specific resistance of *gnp2*Δ to the proline analog 3,4-dehydroproline. Subsequent tests validated that Gnp2 is a specific proline permease in *C. albicans*, which is contrary to the model yeast *Saccharomyces cerevisiae* where proline transport is mediated by four permeases. Furthermore, the induction of *GNP2* appears to be independent of the SPS (Ssy1-Ptr3-Ssy5) regulatory pathway that controls proline utilization in the model yeast, pointing towards rewired proline uptake in *C. albicans*. Additionally, strains lacking *GNP2* were unable to respond to proline-induced filamentation, displayed decreased cytotoxicity to macrophages and showed increased sensitivity to oxidative stress, underlining the importance of proline uptake for fungal virulence. Taken together, the role of Gnp2-mediated proline uptake illustrates the importance of metabolism-driven virulence in *C. albicans*.

183C

Candida albicans infection responses of oral epithelial cells are regulated by glucose transporters.

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Abstract

Background: *Candida albicans* (*Calb*) is a frequent oral mycobiome constituent, but also the most common human fungal pathogen. Oral epithelial cells (OEC) play a key role in coordinating discrimination of commensal from pathogenic states of this fungus. Recent work indicates that metabolic pathways are essential to coordinate these responses. Here, we explore the impact of *Calb* infection of epithelial cells on glucose transporter (GLUT) activity and their role in host responses.

Methodology: We infected OEC cell lines with *Calb* and analysed changes in gene expression of class I/II GLUTs using RT-qPCR. We confirmed increased expression of GLUT1 and GLUT3/14 in patients with chronic hyperplastic candidiasis using immunohistochemistry. We determined the role of these transporters in regulating OEC responses by using specific inhibitors before analysing damage, cytokine secretion, and activation of signalling pathways using ELISA and WB.

Results: Infection with *Calb* induced an increase in class I GLUTs (GLUT1, 3, 14) expression, which was mirrored in patient biopsies. In contrast, class II GLUTs (GLUT9) showed almost complete suppression of expression post-infection. Inhibition of GLUT1 and GLUT3/14 had no impact on cell damage, but resulted in a reduction in G(M)-CSF release and increased c-Fos expression. Further, GLUT3/14 inhibition alone led to decreased MAPK-ERK1/2 and MKP1 phosphorylation.

Conclusion: Modulation of GLUTs expression profiles is a key event in pathogenic infection of OECs by *Calb.* Further, these proteins play a significant role in shaping the subsequent responses of these cells to infection. therefore, they represent potential novel therapeutic targets for mucosal infections.

184A

The role of Grf10 in regulation of copper toxicity in *Candida albicans*

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Abstract

Copper is an essential micronutrient that is toxic at high concentrations. *Candida albicans* maintains copper homeostasis by modulating influx and efflux, and binding it to metallothioneins. The transcription factors Mac1 and Cup2 regulate cellular responses to low and high levels of copper, respectively. We are investigating the role of the transcription factor Grf10 in copper resistance. The *grf10*Δ mutant grows in high (12 mM) copper, but the mechanisms for this resistance are unknown. Resistance was specific to copper, as mutant and WT strains showed equivalent growth with other metals. Resistance to copper was dependent on the dosage of the *GRF10* gene. Restoring the *GRF10* allele in the null strain led to copper sensitivity; however, strains with mutations in a protein-interaction domain of Grf10, *grf10*-D302A and *grf10*-E305A, were resistant to copper, suggesting that Grf10 interacts with another factor to affect copper sensitivity. Using RNA-sequencing, we found 1.5- to 2.5-fold lower expression of genes involved in iron and copper uptake (*FET31, FTR1, CFL2, FRE10* and *FET34)*, and found 1.5-fold increased expression of the *CRD2* copper metallothionein in the *grf10*Δ mutant. There was no difference in the accumulation of copper as measured by ICP-MS, suggesting a difference in bioavailability. Our data are consistent with a model in which Grf10 is necessary for full expression of copper uptake genes, and the resistance to high copper in the *grf10*Δ mutant may be due to decreased uptake and/or increased sequestration.

185B

Sugar phosphorylation controls carbon source utilization and virulence of *Candida albicans*

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Abstract

Candida albicans is an opportunistic human fungal pathogen that relies upon different virulence traits, including morphogenesis, invasion, biofilm formation, nutrient acquisition from host sources as well as metabolic adaptations during host invasion. In this study, we show how sugar kinases at the start of glycolysis modulate virulence of *C. albicans*. Sequence comparison with *S. cerevisiae* identified four enzymes (Hxk1, Hxk2, Glk1 and Glk4) in *C. albicans* with putative roles in sugar phosphorylation. Hxk2, Glk1 and Glk4 demonstrate a critical role in glucose metabolism, while Hxk2 is the only kinase important for fructose metabolism. Additionally, we show that Hxk1 controls *HXK2*, *GLK1* and *GLK4* expression in the presence of fermentable as well as non-fermentable carbon sources, thereby indirectly controlling glycolysis. Moreover, these sugar kinases are important during virulence. Disabling the glycolytic pathway reduces adhesion capacity, while deletion of *HXK1* decreases biofilm formation. Finally, we demonstrate that *hxk2Δ/Δ glk1Δ/Δ glk4Δ/Δ* and *hxk1Δ/Δ hxk2Δ/Δ glk1Δ/Δ glk4Δ/Δ* have attenuated virulence upon systemic infections in mice. These results indicate a regulatory role for Hxk1 during sugar phosphorylation. Furthermore, these kinases are essential during growth on glucose or fructose, and *C. albicans* relies on a functional glycolytic pathway for maximal virulence.
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187B

Identifying the regulatory network controlling the interactions of *Candida albicans* with host mucin

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Abstract

Candida albicans is a commensal member of the human microbiota that is also capable of causing superficial and disseminated infections and is known to be the predominant fungal pathogen of humans in clinical settings. Mucosal linings are critical for protecting the host against infections, and it is the biological and viscoelastic properties of mucus, predominantly attributed to glycoproteins called mucins, that provide this protection. Through largely unknown mechanisms, mucins are involved in maintaining a healthy microbial balance on these linings by directly interacting with microbes. Oftentimes invading microbes colonize mucosal surfaces by forming microbial communities called biofilms. Biofilms can have detrimental impacts on the host because, once formed, they are highly resistant to chemical and mechanical perturbations. We aim to determine the regulators that mediate the ability of *C. albicans* to form biofilms on mucin-coated surfaces. We screened a library of 211 homozygous transcription factor deletion mutants using *in vitro* biofilm assays in the presence of mucin to identify the regulators involved in controlling the interactions with mucin. A total of 12 “hits” were found to be defective or refractory to mucin compared to the wildtype. Using RNA-seq and ChIP-seq, we are mapping the regulatory connections of each transcription factor to one another and to downstream target genes to determine the regulatory network controlling the ability of *C. albicans* to interact with mucin. Understanding how *C. albicans* modulates its interactions with mucin will shed new light on the regulatory control that evolved to mediate this important interaction with the host.

188C

CRISPR-Cas9 mutagenesis and single gene reintegration suggests functional diversity within the *Candida albicans TLO* gene family

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Abstract

Candida albicans has between 10-15 Telomere-associated ORF family *(TLO)* genes, whereas its closest relative, *Candida dubliniensis*, has two. The Tlo proteins are components of the Mediator complex which plays an important role in transcriptional regulation. CRISPR-Cas9 mutagenesis was used to generate a *TLO* null mutant of *C. albicans.* Phenotypic analysis of the mutant showed significantly reduced fitness, with major defects in growth rate, morphogenesis, stress resistance and virulence in a *Galleria mellonella* model. Clade representative *TLOα1, TLOβ2* and *TLOγ11* constructs were reintroduced into the null mutant background to determine if members of the *TLO* gene family exhibit functional differences. The genes were reintroduced under the control of the *TET1* and *ENO1* promoters. *TLOα1* and *TLOβ2* expression restored stress tolerance and growth rate, in some cases to the level of the WT. *TLOβ2* expression also showed a dramatic effect on morphology resulting in constitutive true hyphal growth. Moderate expression of *TLOγ11* had no detectable effect on many of the phenotypes tested, however overexpression increased biofilm formation in Spider medium, and also conferred increased resistance to cell wall stressors. These data suggest that individual *TLO* genes have distinct functions and that the diversity within the *TLO* family may contribute to the relative success of *C. albicans* as a coloniser and pathogen of humans.

189A

Dissecting the mechanisms governing inter-kingdom interactions between *Candida albicans* and *Lactobacillus* species

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Abstract

Interactions between bacteria and fungi are ubiquitous in nature, yet little is known about the mechanisms governing these interactions. In humans, the opportunistic fungal pathogen *Candida albicans* is a common member of the mucosal microbiota that is capable of causing both superficial infections and life threatening systemic disease. Vaginal candidiasis occurs in approximately 75% of healthy women at least once in their lifetime, with fungal overgrowth often developing after a decline in bacterial abundance due to antibiotic use. *Lactobacillus* species are prominent constituents of the vaginal microbial community and the most common industrial probiotic, with current research focusing on exploiting probiotic bacterial species to promote a healthy vaginal microbiome. With the goal of identifying the mechanism(s) by which specific probiotic organisms affect *C. albicans* virulence, we examined the effects of *Lactobacillus* strains on the ability of *C. albicans* to switch from yeast to filamentous morphologies, a cellular transition important for virulence. We observed that several species of *Lactobacillus* secrete a factor that is able to repress *C. albicans* morphogenesis. Bioassay-guided fractionation and subsequent structural elucidation work linked this activity to a defined molecular entity. Functional genomics and selection-based strategies were then leveraged to identify *C. albicans* genes important for this interaction. Our future work aims to further elucidate the mechanisms underlying this inter-kingdom interaction and facilitate the development of optimized probiotics and novel therapeutic strategies.

190B

Albumin enhances vaginal epithelial damage by *Candida glabrata* through modulation of fungal iron metabolism in a host cell-dependent manner

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Abstract

The opportunistic pathogen *Candida glabrata* is the second most frequent causative agent of vulvo-vaginal candidiasis (VVC), a disease that affects 70% of women at least once during their life. Albumin is an abundant protein of the vaginal fluid and increased concentrations are associated with colonisation by vaginal pathogens. Hence, we investigated the effect of albumin on the pathogenicity of *C. glabrata*.

In an *in vitro* VVC model, the influence of albumin on adhesion and damage capacity of *C. glabrata* was analysed. Further, fungal growth and iron homeostasis, as well as albumin uptake and endocytosis by the host cells were investigated.

C. glabrata normally is incapable of inflicting damage to human vaginal epithelial cells, yet the presence of albumin increased *C. glabrata* adhesion and capacity to cause damage. The increased damage correlated with increased fungal growth, which depended on the presence of human host cells. We also observed that epithelial cells take up albumin, and damage by *C. glabrata* in presence of albumin was strongly reduced by inhibiting macropinocytosis. Screening of a *C. glabrata* deletion mutant library showed that Hap5, a key regulator of iron homeostasis, is essential for increased damage in the presence of albumin. Transcriptional analysis showed that iron-acquisition and metabolism are differentially regulated in presence of albumin.

Collectively, we demonstrated that albumin can augment *C. glabrata* growth and damage capacity during interaction with vaginal epithelial cells. This highlights albumin as a potential key player in the pathogenesis of VVC.

191C

Phr1 controls adhesion of *Candida parapsilosis*

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Abstract

We and others have shown that the *C. parapsilosis* adhesin, Als7, mediates adhesion of yeast to human epithelial cells and extracellular matrix under fluid shear. Clinical isolates showed greatly increased adhesion when grown in human plasma or mammalian tissue culture medium 199 (M199), and the mRNA for Als7 was upregulated under these conditions. Here we report that other potential virulence factors are upregulated in M199 and plasma, and that Phr1 plays a major role in adhesion. RNA seq was used to compare the transcriptome of 3 adhesive clinical isolates of *C. parapsilosis* grown in YPD, M199 and plasma. Increased expression of the 12 most strongly upregulated genes was confirmed using quantitative PCR. *PHR1* was one of the most highly upregulated genes in plasma (80-150 fold above YPD) and was strongly upregulated by neutral or basic pH (qPCR). Deletion of *PHR1* by CRISPR-Cas9 resulted in significant loss of adhesion under shear flow. Phr1 is a member of a family of fungal transglycosylases known to act on β-(1,3)-glucans. These results indicate that in addition to increased expression of adhesins, remodeling of cell wall carbohydrates may also be necessary for adhesion of *C. parapsilosis*. Additionally, plasma provides an important host derived cue to *C. parapsilosis* that triggers major changes in its transcriptome that are likely necessary for virulence.

192A

High-throughput identification of genes required for *Streptococcus mutans* biofilm formation in co-culture with *Candida albicans*.

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Abstract

Streptococcus mutans and *Candida albicans* are residents of the oral microflora and opportunistic pathogens. *Streptococcus mutans*, a causative agent of dental caries, has been shown to interact synergistically with *C. albicans* in dual-species biofilms and augment the progression of caries. Identification of *S. mutans* gene products that play critical roles for the growth of dual-species biofilms with *C. albicans* could serve as targets for preventing caries. To identify these genes in *S. mutans*, a pooled transposon (Tn) mutant library of *S. mutans* was grown in mono-culture (control) and co-culture (with *C. albicans*) biofilms on saliva-coated hydroxyapatite discs statically for 18 hours in sucrose rich medium. The Tn mutant library consists of >50,000 mutants, with uniform coverage across all non-essential genes and intergenic regions. We used transposon sequencing to compare the relative abundance of Tn mutants between samples. Using this approach we identified 33 genes that increase or decrease *S. mutans* growth in the presence of *C. albicans*. Transposon insertions in 18 genes were depleted, including genes with functions related to DNA damage, deaminase activity, bacteriocin secretion, and others. Transposon insertions within fifteen genes showed increased abundance in the dual-species biofilms, including elongation factor Tu, uridylate kinase and hypothetical proteins. Our forward genetic screening results show that under the conditions used, only a small (~2%) sets of *S. mutans* genes are required for dual-species biofilm growth with *C. albicans.* Validation of the results with selected *S. mutans* mutants for their effects on biofilm growths with *C. albicans* is in progress.

194C

Resistance to Quinone Mediated Oxidative Stress in *Candida albicans* is Promoted by Four Flavodoxin-Like Proteins

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Abstract

The ability of the fungal pathogen *Candida albicans* to resist oxidative stress is key for virulence. We identified four Flavodoxin-Like Proteins (FLPs; Pst1, Pst2, Pst3, and Ycp4) on the inner surface of the *C. albicans* plasma membrane that mediate an antioxidant function essential for virulence. FLPs combat oxidative stress by promoting the reduction of quinone molecules without formation of toxic semiquinone radicals. Fungi encounter different quinones as metabolic byproducts, plant-derived compounds, and certain pharmaceutical drugs. In this study, we examined the ability of individual FLP genes to detoxify a range of different quinones. The results indicated that PST3 plays a special role in detoxifying small quinones. To determine if this was due to the abundance or the inherent function of each FLP, antibodies were raised to determine FLP protein levels before and after stimulation with quinones. Although these studies suggested that the high level of Pst3 produced after oxidative stress could contribute to its key role in resisting quinones, control studies suggested that Pst3 has distinct functional properties. The FLPs were singularly expressed in a deletion mutant strain using the same promoter to normalize the level of FLP produced. The results showed, that when expressed independently, Pst1 and Pst3 conferred *C. albicans* with the best protection from small quinones. Although *pst3Δ* showed distinct properties *in vitro*, all four FLPs had to be deleted to cause a severe virulence defect in mice. Future studies will focus on the role of FLPs during a time course of infection.

195A

A COMPOSITE NON-TOXIC AMINO ACID SOLUTION INHIBITS AND DISRUPTS BIOFILM FORMATION IN *CANDIDA ALBICANS*

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Abstract

Candida albicans, a commensal yeast of the human microbiota, is considered a pathobiont due to its prevalence as a fungal human pathogen. *C. albicans* infections can manifest as mucosal and systemic infections, and a major contributing factor to these infections is the ability of *C. albicans* to form structured, surface-associated communities of cells called biofilms. Biofilm-related infections are found colonizing many niches of the human body, including mucosal surfaces and implanted or indwelling medical devices. These biofilm-related infections are refractory to many currently available antimicrobial treatment strategies, thus constituting a unique challenge in clinical settings. Here we present an anti-biofilm strategy using a composite, non-toxic amino acid solution that is highly effective at both inhibiting *C. albicans* biofilm formation and disrupting established biofilms. We also provide evidence that the amino acid solution effectively reduces *C. albicans* biofilm cells following 24 hours of treatment. Furthermore, we demonstrate that this solution has anti-biofilm properties against several members of the *Candida* clade, including *C. auris*, in addition to a diverse array of bacterial biofilms. The anti-biofilm efficacy of this amino acid solution across domains of life demonstrates its promising potential for broad-spectrum use in clinical settings.

196B

Role of select extracellular vesicle cargo during C. albicans biofilm growth

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Abstract

BACKGROUND: *Candida* commonly produces disease via biofilm growth. Recent studies identified the importance of extracellular vesicles (EVs) for delivery of the extracellular matrix, protecting the biofilm community from antifungal therapy. We therrized specific EV cargo are responsible for matrix and other biofilm features. **METHODS:** We collected EVs from wild-type and ESCRT mutant *C. albicans* biofilms followed characterization of the vesicle proteome. A total 576 proteins were identified. Machine learning was used to group proteins based on their abundance across wild type and ESCRT mutants, yielding 43 proteins for functional analysis. Null mutants were screened for adherence, mature biofilm formation, drug-susceptibility, matrix production, and dispersion using complementary assays. Wild-type EV add back studies were performed with mutant strains exhibiting biofilm-associated phenotypes to explore vesicle mechanistic specificity. **RESULTS:** We identified more than 20 null mutants with either adherence, drug susceptibility, or dispersion defects. The majority of biofilm associated mutant phenotypes reversed in strains complemented with a wild-type allele or the exogenous addition of wild type EVs. Most drug-susceptible mutants also exhibited matrix defects. We also identified a subset of mutants displaying both matrix and dispersion defects. Most of the mutants with defects across biofilm assays function in polysaccharide modification. **CONCLUSION:** We find that biofilm EV cargo play key roles in several aspects of biofilm pathogenesis. Machine learning approaches were in useful for efficient selection of proteins of importance. The observations demonstrate vesicle delivered extracellular matrix plays a role in both protection of the cellular community from exogenous drug insults and dispersion.

197C

A role of *C. albicans* Rpn4 in regulating antifungal drug susceptibility

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Abstract

The increasing evolution of antimicrobial drug resistance in microbial pathogens that we have witnessed over the last few decades is continuing to have catastrophic consequences for our ability to succeed with advanced medical approaches, such as chemotherapy, transplants or surgery. Azoles are first-line antifungal drugs targeting the biosynthesis of ergosterol, a major sterol component in fungal membranes (1,2). However, resistance and tolerance to azoles have been shown (3). The suggestion is that both these phenotypically-distinct aspects of reduced azole susceptibility could jeopardise treatments in clinical situations. We show that the *C. albicans* transcription factor Rpn4 is required for azole resistance and tolerance. The *rpn4* deletion mutant has reduced resistance and reduced tolerant growth normally present in wild type cells at supra-minimum inhibitory concentration of fluconazole. RNAseq transcriptomics demonstrated that *C. albicans* Rpn4 has a conserved function with *Saccharomyces cerevisiae* as a transcriptional activator of the 26S proteasomal subunits genes and is further involved in the regulation of several metabolic pathways (4). Our data suggests a divergence between *C. albicans* and *S. cerevisiae* with respect to Rpn4-dependent functions in fluconazole susceptibility. Characterisation of Rpn4, other proteostasis-related genes as well as metabolic regulators will be presented, which dissects the roles of these pathways in azole susceptibility of *C. albicans*.

198A

Relevance of seven-transmembrane receptor protein Rta2 in coordinating endoplasmic reticulum stress responses in Candida albicans

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Abstract

C. *albicans* genome has three Rta1-like genes– RTA2, RTA3 and RTA4. This Rta1-like family of proteins may function as novel potential antifungal targets as they are unique to the fungal kingdom and do not have a murine or human homolog. Both Rta2 and Rta4, the downstream effector molecules of calcineurin pathway, are upregulated upon tunicamycin (TM) exposure. Rta2 is requisite to cope with tunicamycin induced ER stress in a Hac1-independent manner (Thomas et al., 2015). Additionally, it also helps the cells to regain ER homeostasis after ER stress by attenuating the unfolded protein response. Considering the fact that ER stress responses are deeply intertwined with the virulence traits, we checked filamentation and in vitro biofilm forming ability of the mutant. *rta2*Δ/Δ cells showed no defect in these aforementioned pathogenic traits. Transcriptional profiling of *rta2*Δ/Δ cells revealed genes enriched for the gene ontology (GO) processes related to biofilm formation, ribosomal biogenesis, cell wall and mitochondrial function. Sequence homology of these Rta proteins revealed the presence of signature sequence, evolutionarily conserved in the extracellular loop of these seven-transmembrane receptor proteins. We show that the signature sequence of Rta2 do have a key role in providing tolerance to tunicamycin and promotes the cells to regain ER homeostasis. This study for the first time shows the relevance of a 7-transmembrane receptor protein Rta2 during ER stress in C. *albicans*, with implications in future antifungal therapy.

199C

A genome-wide to nanoscale view of the human pathogen *Candida glabrata* from the first surface touch to biofilm formation

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Abstract

Background: The pathogenic yeast *Candida glabrata* is capable of adhesion to human mucosa and biofilm formation, key pathogenic traits that allow *C. glabrata* persistent infections.

Methods: Single-cell Force Spectroscopy (SCFS) was applied to characterize the adhesive properties of *C. glabrata* following interaction with medical relevant surfaces, such as plastic surfaces used in catheters or the human vaginal epithelium cell line VK2/E6E7. A genetic screening was then carried out, followed by RNA-sequencing (RNAseq) to characterize the transcriptomic changes occurring in *C. glabrata* after 24h of biofilm formation on polystyrene.

Results: Interactions measured by SCFC, revealed a strong adhesion ability of *C. glabrata* to plastic surfaces, when compared to glass or to human vaginal epithelial cells. The genetic screen implicated the transcription factors CgEfg1 and CgTec1 in biofilm formation, the RNAseq analysis showed that half of the entire transcriptome of *C. glabrata* is remodelled upon biofilm formation, with 40% under the control of these regulators. Although both activate a number of adhesin-encoding genes during biofilm formation, only CgEfg1 was found to be necessary for the full capacity of *C. glabrata* to adhere to biotic and abiotic surfaces by SCFS, playing a role in the initial interaction with both surface and membrane-embedded epithelial cell proteins.

Conclusion: Overall, this work characterizes the nanoscale interactions established between *C. glabrata* and medically relevant surfaces, while establishing the differential role of CgEfg1 and CgTec1 from the initial cell-surface interaction to mature biofilm formation.

200C

Epigenetic Control of Azole Drug Resistance and Pathogenesis via Set1-mediated Histone H3K4 Methylation

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Abstract

Background: Set1-mediated H3K4 methylation is a well-established epigenetic modification. The role of SET domain epigenetic factors involved in fungal drug resistance in *C. glabrata* have not been determined. Our central hypothesis is that SET domain epigenetic factors regulate genes or pathways that govern antifungal drug resistance. This hypothesis was established based on our published observations showing that loss of SET domain-containing genes can alter the efficacy of antifungal drugs in *S. cerevisiae*.

Methods and Results: We have determined that Set1-mediated histone H3K4 methylation is important for drug resistance. To analyze the role of Set1 in altering azole sensitivity in *C. glabrata*, *SET1* was deleted and RNA-sequencing analysis was performed. Loss of Set1 alters azole drug resistance in *C. glabrata* suggesting a conserved epigenetic mechanism exists. In addition, transcript levels of genes encoding *ERG* genes are significantly lower in the *set1Δ* strain upon azole treatment. To determine if the *set1Δ* strain could alter drug resistance *in vivo*, the *G. mellonella* larvae infection model was used. *Interestingly, loss of SET1 significantly decreased C. glabrata pathogenesis in larvae.* Although azole treatment did not increase survival in larvae injected with a *set1Δ* strain, untreated *set1Δ* infected larvae survived similar to larvae treated with azoles but infected with wild-type *C. glabrata.*

Conclusions: Our data demonstrate a new and unexplored role of Set1 in *C. glabrata* in altering azole drug efficacy and pathogenesis. Epigenetic gene regulation of ergosterol production and ergosterol transport are likely key determinates for altering drug resistance *in vivo*.

201A

Using *in vitro* and *ex vivo* models to unravel *Candida auris* infection strategies

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Abstract

Candida species are a major cause of invasive fungal infections, with *Candida albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* being clinically most relevant. Recently, *C. auris* emerged as a new species affecting humans in various regions of the world, causing invasive infections with high rates of clinical treatment failures and problems with nosocomial transmission.

As pathogenicity mechanisms of *C. auris* are largely unknown, we analyzed a set of *C. auris* strains from different clades during interaction with host cells and *in vitro* mimicking the host or environmental situation. Although we observed strain-specific variations in growth, aggregation and antifungal resistance, all *C. auris* strains consistently did show only moderate virulence potential in our infection models with epithelial cells, endothelial cells or human macrophages. On the other hand, we observed robust survival features of *C. auris* under (long-term) environmental conditions, potentially being critical for fungal spreading.

To mimic dissemination *via* the bloodstream during systemic candidiasis, we used an *ex vivo* whole blood infection model. Similar to other pathogenic *Candida* species, *C. auris* is efficiently killed in human blood and shows a species-specific pattern in immune cell association, survival rate and induction of cytokines. Dual transcriptional profiling of *C. albicans*, *C. parapsilosis*, *C. tropicalis, C. glabrata* or *C. auris* and human blood cells during infection indicates a rather uniform host response governed by pro-inflammatory immune responses. Fungal survival strategies were discrete and ongoing comparisons of the species' transcriptomes will help us to place *C. auris* within the pathogenic landscape of *Candida* species.

202B

Dissecting the mechanisms behind intestinal damage and translocation by *Candida albicans*

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Abstract

The opportunistic fungal pathogen *Candida albicans* can thrive on human mucosal surfaces as a harmless commensal. Translocation across the gut barrier into the bloodstream by intestinal-colonizing *C. albicans* is thought to be the main source of disseminated candidiasis, however, the host and microbial mechanisms behind this process are still largely unclear.

An *in vitro* model of intestinal epithelial cells grown in a transwell system was used to determine the association of fungal translocation with epithelial damage and breakdown of barrier integrity. The *C. albicans* cytolytic peptide toxin, candidalysin, was essential for damage of enterocytes and subsequent fungal translocation. However, invasion and low-level translocation also occurred in a candidalysin-independent manner.

To shed more light on *C. albicans* and host genes associated with invasion and translocation, we have performed dual-species transcriptional profiling during intestinal epithelial infection. The fungal response was dominated by a general hyphal transcriptional program. In addition, early adhesion and invasion lead to differential expression of fungal cell wall-related genes, while the later stages of invasion and damage showed differential expression of genes involved in fungal metabolism and filamentation regulation. Fungal-mediated damage seemed to be the major driver of the host response, with differentially expressed genes mainly detected at later time points.

In ongoing work, fungal genes differentially expressed during epithelial infection are cross-referenced with those related to increased or decreased intestinal damage to identify potential commensalism- and virulence-associated genes. Corresponding deletion strains will then be tested in the transwell system and a complex gut-on-chip infection model.

203C

Structural and functional analysis of seven transmembrane receptor protein Rta3 in *Candida albicans*

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Abstract

Background

Rta1(**R**esistance **T**o **A**minocholestrol) family of proteins are unique to fungal kingdom and can be considered as potential therapeutic targets. Sequence homology of these Rta proteins revealed the presence of signature sequence. Published data from our laboratory has implicated Rta3, a 7-transmembrane receptor protein, as the determinant of biofilm development in *C. albicans*. In addition, this protein also plays a role in maintaining the asymmetric distribution of phosphatidylcholine across the plasma membrane. Considering the relevance of Rta3 in *C. albicans*, we aimed to dissect the importance of the conserved signature sequence of Rta3 as well as its C-terminal domain.

Methods

We sought to analyze the functional relevance of Rta3 signature sequence by mutating /deleting conserved residues by using two different mutagenic primer pairs. Flow cytometry and NBD-PC internalization was done as described previously by Hanson et al 2003, followed by fluorescence microscopy.

Results

Herein, we show that the strain lacking the signature motif of Rta3 displayed increased susceptibility to miltefosine, similar to the *rta3*Δ/Δ cells. Consistently, H379R/E380D mutant also displayed increased internalization of NBD-labelled phosphatidylcholine. Additionally the cells expressing **Δ** L979- 1014 (mutant with 12 amino acids deletion in its loop 5 region) also displayed increased susceptibility to miltefosine. Interestingly ΔC 414-464 (C- terminal truncation mutant) behaves similar to the wild-type.

Conclusions

Our data demonstrate the relevance of signature sequence of Rta3 family. In addition,

we offered insight in the Loop5 region of Rta3. These advances may serve as a solid basis for future studies to understand the mechanism of Rta3 mediated miltefosine susceptibility.

204A

Role of autophagy-related proteins during infection of intestinal epithelial cells by Candida albicans.

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Abstract

Background: *Candida albicans* is an opportunistic pathogen causing infections ranging from superficial to the more life-threatening disseminated infections. In a susceptible host, *C. albicans* is able to reach and translocate through the gut barrier, promoting its dissemination in deeper organs. Host countermeasures to limit *C. albicans* colonization and invasion include the secretion of anti-fungal peptides, the secretion of mucus and the recruitment of specific immune cells dedicated to microorganisms clearance. Autophagy is a lysosomal degradation process that contributes to host immunity by eliminating invasive pathogens and modulating the inflammatory response. The protective role of autophagy during *C. albicans* infection is poorly known regarding infection of intestinal epithelial cells. In the present study we investigated the role of the autophagic process during the infection of intestinal epithelial cells by *C. albicans*.

Methods and results: Using immunofluorescent staining against various autophagy-related markers, we demonstrated that key players of the autophagy machinery (LC3, ATG16L1 and WIPI2) are recruited at the *Candida* invasion site. We confirmed these observations by electron transmission microscopy that reported the presence of autophagosomal structures in the vicinity of internalized hypha. The recruitment of autophagy-related proteins to *Candida albicans* entry site is associated to plasma membrane damages. Finally, we used autophagy-deficient epithelial cell lines to demonstrate that autophagy-related proteins contribute to limit invasion and to protects cells from *C. albicans*-induced epithelial cell death.

Conclusions: We have evidenced that autophagy limits invasion and protects epithelial cells from C. albicans epithelial cell death.

205B

The transcription factor Stp2 has stage-specific roles during biofilm development in *Candida albicans*

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Abstract

Candida albicans biofilm cells are shielded from antifungals and host immune response, which is especially problematic for critically ill patients. The increasing thickness and structural complexity of the growing biofilms is paired with nutrient, gas and fluid shifts, leading to constant metabolic rearrangements - from utilization of preferred sugars to alternative carbon sources. Our previous studies demonstrated the importance of *C. albicans* transcription factor Stp2 in utilization of amino acids, a host-abundant nitrogen and carbon source, leading to subsequent hyphal morphogenesis and alkalinization. Since hyphal growth is critical for robust biofilm formation, we investigated whether Stp2 regulates morphological and metabolic adaptation during biofilm development.

The initial adherence and germ tube formation were strongly impaired in a *stp2*Δ mutant, suggesting that Stp2-related processes are important in early biofilms. While mature *stp2*Δ biofilms formed under static conditions had similar density and morphology to the control strains, under continuous flow they had markedly reduced hyphal growth and biomass in all biofilm development stages. Metabolic adaptation became increasingly relevant over time, since the metabolic activity was reduced in mature *stp2*Δ biofilms, leading to increased viability and longevity. The reduced cell lysis was reflected in the relative low abundance of cytoplasmatic proteins in the secretome of *stp2*Δ as compared to the SC5314 biofilms. Importantly, wild-type biofilms showed enrichment in secreted proteases assisting protein breakdown into amino acids, the later identified as the major nutrient source in mature biofilms. Altogether, our data demonstrates the growing importance of amino acid metabolism during *C. albicans* biofilm formation.

206C

Lem3 activity is required for the maintenance of phosphatidylcholine asymmetry across the plasma membrane in Candida albicans.

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Abstract

Background: Biological membrane is made up of lipid bilayers, in which lipids are distributed in an asymmetrical manner between the two leaflets. This asymmetric distribution of phospholipids on the membrane is maintained by balance inward (flip) and outward (flop) translocation of phospholipids across the membrane. ATP-driven phospholipid translocases, P4-ATPases regulate this asymmetric distribution by translocating phospholipids from the exoplasmic leaflet to the cytoplasmic leaflet of the plasma membrane. In S.*cerevisiae* Lem3 is identified as beta subunit of Dnf1, Dnf2 that regulates the transport of phosphatidylethanolamine, phosphatidylcholine and monohexosyl glycosphingolipids. Parallel studies on P4-ATPases and their cognate non-catalytic partners are limited in the pathogenic fungus C.*albicans* and so we were prompted to elucidate the role of Lem3 in this fungus.

Methods: *SAT*-flipper strategy was used to delete Lem3 from the wild type strain. NBD-PC internalisation assay and flippase activity was performed as described in Srivastava et al., 2017. Results: Herein, we have found that loss of Lem3 results in decreased susceptibility to the alkylphosphocholine drug miltefosine. Apparently the internalization of NBD-labelled phosphatidylcholine decreased, as well as the PC-specific flippase activity was abrogated. Disruption of Lem3 function did not affect susceptibility to cell wall damaging agents. Virulence traits such as hyphae morphogenetic was reduced while in vitro biofilm formation remained unchanged. Despite the reduced hyphal growth of the *lem3* Δ/Δ mutant in vitro, virulence was not attenuated in vivo mice model of systemic infection.

Conclusion: Our data demonstrates that Lem3 regulates the transbilayer movement of phosphatidylcholine across the plasma membrane in C.*albicans* .

207A

Repeated selection of metal transport pathways in multiple Candida dominant Cystic Fibrosis infections

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Abstract

Cystic fibrosis (CF) affects over 70,000 people worldwide, and mucus buildup makes the CF lung highly susceptible to infection by bacteria and fungal species. In three separate CF patients, *Candida lusitaniae* outcompeted existing bacterial infections to become the dominant microbe, and these *C. lusitaniae* populations had significant heterogeneity within the CF lung environment. The only gene mutated among all three populations encodes the mitochondrial high-affinity iron transport protein Mrs4. Replacement of mutated alleles with the reference allele demonstrated each mutation results in varying degrees of function loss. Growth in the presence of Co2+ and Cd2+ confirmed *Δmrs4* mutants respond similarly to the Mrs4 ortholog knockout in *Saccharomyces cerevisiae*, gaining cobalt resistance and cadmium sensitivity. Comparison of mutant alleles to the reference demonstrated loss of function alleles caused a decrease in acidification, potentially due to decreased fermentative metabolism or increased acetate consumption. In the course of these pH assays we observed that growth of *MRS4* mutants in the presence of chromogenic pH indicators resulted in the loss of color through desulfonation. These mutations suggest adaptive alterations in sulfonate utilization and increased sulfur assimilation. Our metabolomics studies have also shown increased levels of taurine in the CF lung, which may act as an alternative sulfur source. These results demonstrate the role of *MRS4* in *C. lusitaniae's* metabolism, and sequencing of later timepoints shows the preservation of multiple alleles within the population, indicating that having different alleles may result in changes in metabolism that promote the survival of *C. lusitaniae* within the CF lung.

208B

Pathogenicity-related traits in *Candida albicans* are influenced by Ire1-dependent homeostatic adaptation to endoplasmic reticulum stress

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Abstract

Background

Unfolded protein response (UPR) is a series of adaptive responses facilitating the management of endoplasmic reticulum (ER) stress to maintain cell homeostasis. Classical UPR pathway works via sensing abrupt protein load by ER transmembrane sensor; Ire1 following *HAC1* splicing and successful Hac1 translation that upregulates various stress-responsive genes. Role of UPR is established in yeast and different pathogenic fungi however remains unelucidated in *C. albicans*.

Methods

The *ire1* DX strain used herein was obtained from a study by Woolford et al, 2016 and complementation mutant was constructed in our laboratory (Ganguly and Mitchell, 2012). Ire1 kinase and nuclease domain deletion mutants were constructed by using mutagenic primer pairs. RT-PCR and qPCR were employed for estimating *HAC1* splicing with gene-specific primers. Cell wall changes were analysed by HPLC and immunoblotting. Effect on pathogenesis was analyzed by inspecting virulence-associated traits in different conditions.

Results

Current study proposes that Ire1 administers *HAC1* splicing and kinase and nuclease activities of Ire1 are critical for successful UPR in *C. albicans*. Additionally, Ire1 has an essential role in variable cellular processes like cell wall damage response, biofilm formation and tolerance to different antifungals. The conclusion is supported by increased susceptibility of *ire1* DX to different cell wall/membrane stressors, compromised filamentation and biofilm formation. *ire1* DX also displayed attenuated virulence in mice advocating a requirement of a functional UPR pathway for survival and proliferation in host.

Conclusion

This study provides a novel insight into UPR mechanism of *C. albicans* and proposes a direct or indirect involvement of Ire1 in pathogenesis of this fungus.

209C

The Role of *Candida albicans* Secreted Polysaccharides in Augmenting *Streptococcus mutans* Adherence and Mixed Biofilm Formation: *In Vitro* and *In Vivo* Studies

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Abstract

The oral cavity is a complex environment harboring diverse microbial species that often co-exist within biofilms on oral surfaces. Within a biofilm, inter-species interactions can be synergistic in that the presence of one organism generates a niche for another enhancing colonization. Among these species are the fungal pathogen *Candida albicans* and the bacterial species *Streptococcus mutans,* the etiologic agents of oral candidiasis and dental caries, respectively. Recent studies have reported enhanced prevalence of *C. albicans* in children with caries indicating potential clinical implications for this interaction. In this study, we aimed to specifically elucidate the role of *C. albicans*-derived polysaccharide biofilm matrix components in augmenting *S. mutans* colonization. Results from *in vitro* studies demonstrated significantly enhanced *S. mutans* retention in mixed biofilms with *C. albicans.* Further, *S. mutans* single species biofilms were enhanced when supplemented with purified *C. albicans* biofilms matrix material, or when grown in cell-free *C. albicans* spent biofilm media, but not upon enzymatic digestion of polysaccharides in spent media. The enhanced *S. mutans* biofilms mediated by the various *C. albicans* effectors was also demonstrated using confocal laser scanning microscopy. Importantly, using a clinically-relevant mouse model of oral co-infection, analyses of harvested tissue and scanning electron microscopy demonstrated *C. albicans*-mediated enhanced *S. mutans* colonization on teeth and tongues of co-infected mice compared to mice infected only with *S. mutans*. Collectively, these findings strongly indicate that secretion of *C. albicans* cell wall polysaccharides in the oral environment may impact the development of *S. mutans* biofilms and potentially dental caries.

210A

A variant *ECE1* allele contributes to reduced pathogenicity of *Candida albicans* during vulvovaginal candidiasis via altered secretion of candidalysin

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Abstract

Background:

Candida albicans is the primary etiological agent of vulvovaginal candidiasis (VVC) and exerts its pathogenicity through secretion of the peptide toxin candidalysin encoded by the *ECE1* gene. A highly conserved variant *ECE1* sequence exists across a diverse set of clinical isolates. Thus, we sought to determine the relative pathogenicity and mechanism(s) associated with this alternative *ECE1* allele.

Methods:

Isogenic strains harboring WT or variant *ECE1* sequences were engineered in an Δ/Δ*ece1* background. After confirmation of equivalent expression by qPCR, pathogenicity of strains were tested using in vitro epithelial cell and in vivo VVC models of infection and LDH, IL-1β, neutrophil levels monitored. Follow up studies using synthetic candidalysin peptide were also performed. Lastly, a panel of *ECE1* chimeras were constructed to assess potential processing defects and detected by a novel HiBiT-tagging approach.

Results:

Strains transformed with either the variant full length *ECE1* or candidalysin allele, as compared to the WT sequence, demonstrated significantly reduced immunopathogenicity during in vitro or in vivo infection despite equivalent fungal burden. Interestingly, epithelial challenge with WT or variant synthetic peptide revealed similar capacity to elicit damage and IL-1β. Allele profiling and *ECE1* chimera experiments demonstrated that defects in pathogenicity are at least partly due to inefficient *ECE1* processing at the peptide 2-peptide 3 junction.

Discussion:

The *ECE1* gene displays conserved polymorphisms that alter candidalysin secretion and strain pathogenicity. Future work is focused on determining specific amino acid sequences that contribute to these affects across clinical isolates and disease states.

211B

Evolutionarily acquired insertion sequences in the Candida proteome are required for adaptation and virulence

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Abstract

Using a forward genetics screen, we identified an uncharacterized gene orf19.2500 whose loss caused *C. albicans* to lose viability on alternative carbon sources and made drug-resistant biofilms detach early in their developmental program. Biochemical assays showed orf19.2500 is a conserved mitochondrial protein, required for the assembly and activity of the NADH ubiquinone oxidoreductase Complex I of the respiratory electron transport chain, and was thereby named *NDU1*. The C. albicans NDU1 protein is unique, because it is distinguished by three evolutionarily acquired stretches of amino acid insertion sequences, absent from all other eukaryotes except ascomycete fungi belonging to the CTG-clade. Three-dimensional (3-D) protein modeling revealed that two inserts lie in close proximity, and represented the functional hub for respiration, immune evasion, virulence, and potentiating antifungal drug resistance. Our study is the first report on a protein that sets the Candida-like fungi phylogenetically apart from all other eukaryotes, based solely on evolutionary “gain” of new amino acid inserts that are indispensable for function. We have now discovered that >15% of the Candida proteome possess supplemental amino-acid inserts that are CTG clade specific. E.g. comparison of 72 glycosyltransferase proteins across 70 eukaryotic genome databases (21 CTG-yeast + 49 eukaryotic genomes including humans), revealed that 39% of the glycosyltransferase proteins possessed insertion sequences, exclusively in CTG-clade yeasts. Similarly, out of 219 conserved eukaryotic mitochondrial biogenesis proteins, 11% harbored CTG-clade specific inserts. Ongoing studies are focused on understanding the global impact of evolutionarily acquired insertion sequences in the adaptation and virulence of Candida.

212C

Overexpression of Hal22 in *Candida albicans* influences virulence in a *Galleria mellonella* model of infection

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Abstract

Filament formation is a major virulence trait of *Candida albicans.* We previously identified a subset of proteins that appeared to be involved in the transition from yeast cells to the filamentous form. The levels of these proteins change at a point of high protein flux and their depletion may be required for filamentation to proceed. Hal22 is a protein from this subset which is uncharacterized in *Candida albicans.* To further investigate the relevance of this protein we overexpressed the protein in a wild-type background and characterized the impact on the ability to filament under various inducing conditions along with examining the ability to form biofilms, and the virulence in a *Galleria mellonella* model of infection. Mutant strains of Hal22 in S. cerevisiae show altered sensitivity to environmental stressors, so we examined these in our over-expression strain as well. We demonstrate a significant attenuation of virulence, altered biofilm formation and caffeine sensitivity. However, unlike *S. cerevisiae*, we have not been able to demonstrate a link between this protein and salt tolerance. This suggests a role in *C. albicans* virulence and perhaps divergence in function compared to the *S. cerevisiae* version of the protein.

213A

Over-expression of a *Candida albicans* gene downregulated during filamentation alters virulence but not hypha induction

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Abstract

Numerous transcriptional and post-translational changes occur during *C. albicans* hyphal induction. Filamentation is necessary for virulence traits such as biofilm formation and damage to host tissues. Previous studies in our lab have identified certain proteins whose presence may negatively influence the transition to filamentation. Our current understanding of the function of these proteins is limited and additional investigation is needed to fully understand the relationship between their expression levels and the suppression or development of the filamentous forms. We have taken the approach of over-expressing some of the recently identified proteins under conditions that promote filamentous growth of *C. albicans* and found varied effects on morphology. The product of the uncharacterized gene *C1_05990C_A* is part of this group and here we show the effect of over-expression of the gene on a wild-type strain. We examined growth in a range of hypha-inducing media, in the presence of several chemical stressors and virulence in the *Galleria mellonella* model of infection. The overexpression strain shows decreased virulence in the *Galleria mellonella* model but appears to be largely unaffected by other conditions. The strain also did not appear to show the decreased rate of vegetative growth observed in *Saccharomyces cerevisiae.* We are continuing to examine the *in vitro* role of this gene to better understand the mechanism behind this *in vivo* result.

214B

The *Candida albicans* Telomeric Associated ORF (TLO) gene family is implicated in tolerance to fluconazole

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Abstract

Candida albicans is an opportunistic cause of oral infection. Development of resistance and tolerance to antifungal drugs is a growing concern. This study investigated the role of the *TLO* gene family in the response to the azole drugs, which inhibit fungal sterol biosynthesis. The *TLO* family is comprised of 14 genes which encode Med2, a subunit of the multiprotein Mediator complex, which is involved in the global control of transcription in all eukaryotes.

A *TLO* deficient mutant of *C. albicans* in which all 14 genes were deleted was generated using CRISPR-Cas9 technology. Representative *TLO* genes were reintroduced to complement the deletions. RNA-Seq analysis was used to compare the strains when grown in YPD at 37°C for 4 hours. Expression of *TLO* genes was examined by qPCR. Fluconazole MICs were investigated using E-tests and microdilution assays, and the membrane sterol content identified using mass spectrometry.

Drug sensitivity assays revealed increased tolerance of the *TLO* null mutant to fluconazole compared to the parent strain, which was reversed upon reintroduction of representative *TLO* genes. mRNA transcripts of the *TLO* genes were reduced in the parent strain upon exposure to fluconazole. RNA sequencing data suggested changes in cell wall organisation and reduced expression of sterol biosynthesis genes in the *TLO* null mutant. Mass spectrometry revealed reduced ergosterol content in the *TLO* mutant cell membrane.

These data confirm the involvement of Tlo proteins in the development of *C. albicans* tolerance of azole drugs, highlighting their potential as targets for the development of novel antifungal drugs.

215C

Characterization of the mechanism of action of the *Enterococcus faecalis* bacteriocin EntV on *Candida albicans*

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Abstract

Candida albicans shares communal niches with multiple bacterial species. Previous work from our group demonstrated that the Gram-positive bacterium *Enterococcus faecalis*, a normal constituent of the oral and gut microbiome that is often co-isolated with *C. albicans*, antagonizes hyphal morphogenesis, biofilm formation, and virulence in *C. albicans.* These effects are mediated by EntV, a bacteriocin and antimicrobial peptide produced by *E. faecalis*. The main aim of this work is to unveil the molecular mechanism behind the activity of EntV on *C. albicans*. Using fluorescence microscopy, we determined that EntV binds to the cell walls of several *Candida* species, including both yeast and hyphae of *C. albicans*. Contrary to other antimicrobial peptides, it does not cause cell lysis and does not synergize with cell wall damaging agents. Moreover, we screened a library of *C. albicans* mutants for strains with altered susceptibility to the peptide; most of the positive hits had functions related to cell wall maintenance and were further screened to ascertain changes in the staining patterns. Furthermore, to identify the target layer on the cell wall, pull-down assays were performed. Mannan was identified as the major wall component able to bind the peptide. Finally, live imaging of macrophages incubated with *Candida* was carried out in order to assess any change in the phagocytic behaviour in presence of the peptide. Identifying the molecular target of EntV in regard to the anti-virulence mechanisms of *C. albicans* is an important step in its further development as a therapeutic addition to the classical antifungal agents.

216A

Short peptides derived from EntV inhibit virulence and biofilm formation in *Candida albicans*

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Abstract

Candida albicans exists as a member of the commensal flora of the skin and gut where many complex polymicrobial interactions occur with genera such as Pseudomonas, Staphylococcus, and Streptococcus. Some of these interactions potentiate or inhibit virulence. The bacterial gastrointestinal commensal species Enterococcus faecalis produces a small peptide, EntV, that modulates C. albicans virulence. The active 68 amino acid EntV peptide inhibits biofilm formation in vitro; biofilm-related infections are difficult to treat with current therapeutics. EntV also attenuates fungal virulence in a Caenorhabditis elegans infection model and a murine oral candidiasis model. We sought to identify the regions of EntV responsible for the anti-fungal activity, and based on structural information, we hypothesized that it could be localized to a single helix of the mature peptide. In this study, we report that smaller peptides derived from this helix ranging from 12 to 16 amino acids have equal to improved efficacy in inhibiting C. albicans virulence and biofilm formation. These smaller peptides attenuate virulence in the C. elegans infection model, inhibit initial adhesion to abiotic surfaces, and reduce the size of mature biofilms measured by confocal microscopy. Further trimming of these peptides to fewer than 11 amino acids reduces and eventually eliminates activity. These data indicate that EntV-derived peptides warrant further investigation as potential non-fungicidal additives to medical devices and antifungal therapeutics.

217B

Negative regulation of biofilm development by the CUG-Ser1 clade-specific histone H3 variant is dependent on the histone chaperone CAF-1 complex in Candida albicans

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Abstract

Morphological plasticity is one of the key pathogenic attributes of *Candida albicans*. A complex transcriptional circuit is known to regulate morphological transitions in response to specific environmental cues in this organism. We previously described a CUG-Ser1clade-specific histone H3 variant ($H3V^{CTG}$), that differs from its canonical form by three amino acids, as a major negative regulator in planktonic to biofilm growth transition (Rai et al. 2019). CAF-1 and HIRA are the two well conserved chaperone complexes involved in the incorporation of histone H3 in humans in replication coupled (RC) and replication independent (RI) manner respectively. The variant specific amino acid residues of histone H3 variants act as distinct chaperone recognition sites in humans. In order to identify the chaperones involved in biased $H3V^{CTG}$ loading into chromatin in *C. albicans*, null mutants of subunits of CAF1 and HIRA were generated. Solid surface filamentation and biofilm assays reveal that mutants of subunits of the CAF-1 chaperone complex mimic the phenotype of the $H3V^{CTG}$ null mutant. The occupancy of $H3V^{CTG}$ is found to be reduced at the promoters of biofilm genes in the absence of CAF-1 subunits. We hypothesize that CAF-1 is a putative $H3V^{CTG}$ chaperone. These findings illustrate the acquisition of an unconventional function in morphogenesis by CAF-1 in *C. albicans*.

218C

Comparative analysis of *Candida glabrata*, *Candida tropicalis* and *Candida parapsilosis* extracellular vesicles produced by planktonic cells and biofilms

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Abstract

Several species of fungal pathogens, including *Candida albicans*, produce extracellular vesicles (EVs) abundantly packed with diverse cargo including proteins, lipids, nucleic acids, carbohydrates and other molecules. Candidial EVs, in addition to their physiological role and involvement in intraspecies communication, might be also responsible for the interactions of fungi with host cells during the infection and can contribute to fungal virulence. Thus far detailed information only on *C. albicans* EVs were available, and the data on EVs produced by non-albicans *Candida* species of clinical importance were significantly missing. Therefore we have performed the isolation and characteristic of EVs produced by *C. glabrata*, *C. tropicalis* and *C. parapsilosis* planktonic cells and biofilms in order to compare these structures. Candidial EVs were obtained with differential ultracentrifugation and defined as a heterogeneous population of structures with diameters ranging from 60 nm to 300 nm and the differences in size distribution between EVs obtained from planktonic forms and biofilm-derived vesicles were observed. The determined total content of vesicular proteins, carbohydrates and phospholipids also indicates the significant variability between EVs produced by biofilm or planktonic cells. The proteomic identification of individual molecules carried by candidial EVs indicates that vesicles may contain numerous virulence factors, enzymes involved in cell wall remodeling, membrane transporters and enzymes of cytoplasmic origin, which suggests the important role of EVs both in the biology of these fungi and in the pathogenesis of candidial infections.

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219A

Leveraging a comprehensive essentiality prediction model to define the function of fungal-specific essential genes

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Abstract

Human fungal pathogens impose a profound medical and economic burden on society; however, effective antifungal therapeutics are in extreme shortage. This is in part due to the emergence of resistance coupled to the fact that fungi and humans are both eukaryotic, minimizing the number of divergent cellular targets to be exploited for therapeutic intervention. The fungal pathogen *Candida albicans* persists as the primary cause of systemic mycotic infections in North America, with mortality rates at ~40%, despite treatment. The majority of antimicrobial agents in clinical use target functions essential for pathogen viability, making essential genes attractive candidates for antifungal development. Functional genomic analysis in *C. albicans* provides unprecedented power for identifying new therapeutic targets, however, a comprehensive assessment of gene essentiality in this species has yet to be conducted. Here, we employed the GRACE (Gene Replacement and Conditional Expression) mutant library, covering ~40% of the *C. albicans* genome, and developed a machine learning model to make essentiality predictions for 6,638 genes annotated in the *C. albicans* genome. We assessed the accuracy of our computational model by constructing ~800 new GRACE strains and testing them for essentiality. Integrating our machine learning model with co-expression datasets, we defined the function of several previously uncharacterized genes including one involved in kinetochore function (*C1_01070C_A*), one important for mitochondria function (*C6_03200W_A*), and one involved in translation initiation (*C2_04370W_A*). Thus, this work provides the most comprehensive prediction of gene essentiality in *C. albicans* and assigns function to several previously uncharacterized genes.

220B

Genetic Analysis of Sirtuin Deacetylases in Hyphal Growth of *Candida albicans*

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Abstract

Candida albicans is a major human fungal pathogen that encounters varied host environments during infection. In response to environmental cues, *C. albicans* switches between ovoid yeast and elongated hyphal growth forms, and this morphological plasticity contributes to virulence. Environmental changes that alter the cell's metabolic state could be sensed by sirtuins, which are NAD^+-dependent deacetylases. Here we studied the roles of three sirtuin deacetylases, Sir2, Hst1, and Hst2, in hyphal growth of *C. albicans*. We made single, double, and triple sirtuin knockout strains and tested their ability to switch from yeast to hyphae. We found that true hyphae formation was significantly reduced by the deletion of *SIR2* but not *HST1* or *HST2*. Moreover, the expression of hyphal-specific genes *HWP1*, *ALS3*, and *ECE1* decreased in the *sir2Δ/Δ* mutant compared to wild-type. This regulation of hyphae formation was dependent on the deacetylase activity of Sir2, as a point mutant lacking deacetylase activity had a similar defect in hyphae formation as the *sir2Δ/Δ* mutant. Finally, we found that Sir2 and Hst1 were localized to the nucleus, with Sir2 specifically focused in the nucleolus. This nuclear localization suggests a role for Sir2 and Hst1 in regulating gene expression. In contrast, Hst2 was localized to the cytoplasm. In conclusion, our results suggest that Sir2 plays a critical and non-redundant role in hyphal growth of *C. albicans*.

221C

Conducting genetic interaction analysis with CRISPR-Cas9-based strategies in *Candida albicans*

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Abstract

Candida albicans are an opportunistic fungal pathogen found in the oral mucosa, the gut, the vaginal mucosa, and humans' skin. While *C. albicans* can cause superficial infections, severe invasive infections can occur in immunocompromised individuals. Understanding the survival mechanisms and pathogenesis of *C. albicans* is critical for novel antifungal drug discovery. Determining the relationships between different genes can create a genetic interaction map, which can identify complementary gene sets, central to *C. albicans* survival, as potential drug targets in combination therapy. A genetic approach using the CRISPR-Cas9-based genome editing platform will focus on genetic interaction analysis of *C. albicans* stress response genes. The ultimate goal is to create a stress response gene deletion library to study its pathogen survival role. This library of single and double stress response gene mutants will be screened under diverse growth conditions to assess their relative fitness. Genetic interaction analysis will help map out epistatic interactions between fungal genes involved in growth, survival, and pathogenesis and uncover putative targets for combination antifungal therapy based on negative or synthetic lethal genetic interactions.

222A

Candida parapsilosis virulence attributes expression are repressed by *NDT80* transcription factor

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Abstract

Among non-albicans species, *Candida parapsilosis* is one of the most prevalent specie causing invasive candidiasis in immunocompromised patients. Its prevalence results from its notorious capacity to persist in hospital environment, due to its ability to adhere to biotic and abiotic surfaces and to form biofilm. In *C. parapsilosis*, *NDT80* gene (*CPAR2-213640*) was associated with biofilm formation, however, its mechanistic role remains to elucidate. Here we show that deletion of *NDT80* gene substantially changed *C. parapsilosis* colony and cell morphologies, from smooth and yeast-shaped to crepe and pseudohyphal/elongated forms. Adherence to polystyrene microspheres and biofilm formation were enhanced in both *ndt80Δ* and *ndt80ΔΔ* mutants, comparatively to wild type strain. Additionally, we identify *NDT80* as a repressor of adhesin Als7, protein kinase Mkc1 and Ume6, Cph2, Cwh41, Ace2 and Bcr1 transcription factors. Interestingly, *ndt80ΔΔ* mutant, in its spontaneous pseudohyphae phenotype, promptly adhere to macrophage, efficiently inducing its phagocytosis to ultimately provoke macrophage disruption. Our findings clearly identify Ndt80 as a repressor of *C. parapsilosis* virulence attributes.

225C

Lactobacillus rhamnosus antagonizes *Candida albicans*' pathogenicity by altering its gene expression and metabolism

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Abstract

Removal or imbalance of the intestinal bacterial microbiota by antibiotic treatment can initiate fungal overgrowth – a significant predisposing factor for disseminated candidiasis in immunocompromised patients. As probiotics, lactobacilli are well known for their protective potential against *Candida albicans*. Using a static *in vitro* gut model and an intestine-on-chip model, we were able to demonstrate a protective effect of *Lactobacillus rhamnosus* against *C. albicans* pathogenicity. We observed bacteria-induced shedding and reduced fungal burden as potential mechanisms to mediate protection. To elucidate the molecular events during the complex interactions between *L. rhamnosus*, *C. albicans,* and intestinal epithelial cells (IECs) in detail, the transcriptional profiles of *C. albicans* and overall metabolic changes in this model were assessed. Colonization of IECs with *L. rhamnosus* caused down-regulation of several *C. albicans* genes indispensable for virulence during infection. This protective potential of *L. rhamnosus* relies on its growth, which is sustained by IECs. Metabolome analysis of supernatants identified distinct metabolites produced by IECs that sustain bacterial growth. These metabolites also promoted *L. rhamnosus* growth independent of IECs. Besides, *L. rhamnosus* colonizing host cells alters the metabolic environment by consuming various nutrients and by secretion of specific metabolites. Some of these can reduce *C. albicans*-induced cytotoxicity.

Collectively, using transcriptomics and metabolomics, the complex interactions of *C. albicans*, antagonistic bacteria, and the host epithelial barrier were dissected. These insights could serve as a stepping stone for the utilization of lactobacilli or their secreted metabolites to prevent the development of disease.

226A

Modeling skin colonization of Candida auris, an emerging multi-drug resistant human fungal pathogen

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Abstract

Background: Four distinct clades of *Candida auris* have emerged since its identification as a human fungal pathogen in 2009. Of increasing concern, *C. auris* has evolved resistance to all major classes of antifungal drugs. *C. auris* colonizes patient skin and persists on environmental surfaces, enabling transmission in healthcare facilities.

Methods: We established a clinically relevant murine model of *C. auris* skin colonization, longitudinally testing surface and full-thickness skin. We assessed immunologic, iatrogenic and environmental perturbations to investigate risk factors for colonization, including diet/diabetes, immune deficiency and antibiotic/antifungal receipt. We tested commonly used skin-directed antiseptics, e.g. chlorhexidine, to determine if they protected or promoted *C. auris* colonization.

Results: *C. auris* is primarily a skin colonizer, compared with *C. albicans*, a gut colonizer. The four distinct clades of *C. auris* differentially colonized mouse skin, consistent with human epidemiologic findings. While skin surface tests negative days after colonization, we discovered that *C. auris* resides deeper within cutaneous tissue for months. Lymphoid cell-immunodeficient mouse models have prolonged *C. auris* colonization on skin and other sites, with no signs of infection. Chlorhexidine could protect mice from being colonized with *C. auris* and serves to decolonize mouse skin.

Conclusion: We present the first murine *C. auris* skin colonization model. Our discovery of fungal residence within skin tissue has implications for infection control in human populations. Immune deficiency is a risk factor for prolonged *C. auris* colonization. Chlorhexidine should be tested clinically for its protective and decolonizing efficacy against *C. auris*.

227B

Candida albicans Pho84, the high affinity phosphate transporter, participates in positive antifungal feedback.

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Abstract

Candida albicans cells that lack the phosphate transporter Pho84 are attenuated in virulence and hypersensitive to oxidative stress. *PHO84* expression is induced during the interaction with the host. *In vitro,* Pho84 is rapidly expressed and trafficked to the cell surface when cells' environment changes from high to low ambient Pi, at time points before depletion of intracellular phosphate stores could occur. Appearance of Pho84 at the cell surface was delayed in the presence of inducer of superoxide oxidative stress, plumbagin. We determined that for plumbagin exposure, the delay in Pho84 appearance occurred at the level of transcription. This delay was rescued by co-exposure to the reactive oxygen species (ROS) scavenger N-acetylcysteine. Cells with genetically or pharmacologically reduced Pho84 activity are hypersensitive to amphotericin for growth, and induction of intracellular ROS by amphotericin inversely corresponds to the *PHO84* gene dosage. Exposure to amphotericin B also delayed Pho84 appearance. Amphotericin and Pho84 inhibitors may mutually potentiate their anti-candidal activity by a positive feedback mechanism: amphotericin exposure could delay and hence reduce Pho84 expression, thereby sensitizing the Candida cell to Pho84 inhibitors, while Pho84 inhibitors may potentiate ROS induction by amphotericin. Pho84 has no human homolog and phosphate (Pi) homeostasis is fundamentally different between fungi and animals, so small molecule inhibitors of Pho84 may be non-toxic to humans.

230B

Decoding Design Principals of *Candida albicans* Response to Phagosome-Centric Perturbations Via Integrative Systems Biology Approach

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Abstract

Candida albicans (C.albicans) have evolved smart ways to adapt and survive inside phagosome. Deciphering system attributes critical for their adaptation skills is crucial for drug-discovery processes. However, cellular stress response mechanisms are complex, dynamic and highly nonlinear to dissect using traditional reductionistic methods alone. An inter-disciplinary systems biology approach is essential to address such a Grand Challenge. We use qualitative reasoning, mathematical modeling and data analysis techniques, along with data from targeted experiments, to delineate the design principles of molecular networks that govern *C.albicans* response to phagosome-centric combinatorial perturbations. To be more specific, we have developed and validated a grand mathematical model that (i) predicts the time and dose response of key anti-oxidant systems (namely, Catalase, Glutathione and Thioredoxin), protein-thiol pools, relevant transcriptome/proteome to physiologically relevant conditions (ii) elucidates the existence of previously unknown states of key transcription factors (Cap1, Hog1, Cta4 and Mnl1) and cross-talks mechanisms, (iii) underpins the mechanistic basis for stress-cross protection and the observed non-additive dynamics during combinatorial perturbations, (iv) distinguishes the hierarchical role of the cellular antioxidant systems (namely, Cta1, Yhb1, GSH and Trx), and (v) provides a means to justify life/death decisions of a *C.albican* that is engulfed by a phagocyte. On a wider context, the proposed model will enable us to understand the cellular importance of the second messengers (ROS and RNS), evaluate their therapeutic potential, predict the outcome of the host-candida interactions and, more importantly, compare the virulence mechanisms of diverse range of pathogens that are evolutionarily similar.

231C

Increasing rates of Candida parapsilosis (Cp) endocarditis associated with injection drug use (IDU).

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Abstract

Background: IDU has been linked to Candida bloodstream infections (BSI) and endocarditis. Cp forms biofilms that may aid endocarditis pathogenesis. We hypothesized that Cp endocarditis complicating BSI was increasing at our center as a result of IDU.

Methods: We retrospectively reviewed Cp BSI, and defined endocarditis by Duke criteria and physician impressions.

Results: From 2010-2018, there was significant increase in Cp endocarditis (p=0.048), but not Cp BSI (mean: 13/year). Endocarditis was diagnosed in 22% (12/54) of Cp BSI patients in 2015-2018, involving aortic (8), mitral (4), tricuspid (2), pulmonic (1) valves, and pacemaker lead (1). Endocarditis patient median age was 52.5 years; 70% were men. 50% (6/12) had systemic emboli (4 splenic, 2 CNS, 2 limb, 1 renal). Independent predictors of endocarditis were active IDU (OR: 3.8, p<0.001) and prosthetic valve (OR: 2.9, p=0.006). Infection drugs were heroin (7), buprenorphine/naltrexone (2), cocaine (1). 42% (5/12) of patients were treated with liposomal amphotericin B+flucytosine (4) or voriconazole (1), 33% (4/12) with high-dose caspofungin, and 25% (3/12) with other regimens. Median induction therapy was 41.5 days. 50% (6/12) underwent valvular surgery/device removal. 30-day and hospital discharge survival were 83% (10/12) vs. 71% (30/42; p=0.7) and 64% (23/42; p=0.3), respectively, for non-endocarditis Cp BSI.

Conclusions: Cp BSI is increasingly complicated by endocarditis, in particular in association with IDU or prosthetic valves. Cp BSI and Cp endocarditis mortality are comparable. Valve surgery is recommended for endocarditis when feasible, but some patients may be treated successfully with long-term induction and maintenance antifungals.

232A

Four cell surface phosphate transporters in Candida albicans contribute to homeostasis at distinct ambient pH and phosphate concentrations

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Abstract

Phosphate is an essential macronutrient, needed for cells to initiate glycolysis, conduct oxidative phosphorylation, replicate DNA, and produce membranes. We previously found that in *Candida albicans*, the high-affinity inorganic phosphate (Pi) transporter Pho84 activates TORC1 signaling in response to Pi, and is required in oxidative stress responses and cell wall integrity. We here characterize *C. albicans'* 4 known cell surface Pi transporters, encoded by *PHO84*, *PHO89*, *PHO87* and *FGR2*, using single and triple mutants in these transporters. Pho84 is the most important, as its presence alone sustains cellular growth and Pi uptake over the widest range of pH and Pi concentrations. The other predicted high-affinity Pi transporter, Pho89, supports cellular growth in low ambient Pi in alkaline conditions, and in high Pi under all conditions. A triple mutant with intact *PHO89* efficiently takes up Pi between pH 6 and 9, while its uptake velocity abruptly slows at pH≤5. The predicted low affinity transporters Pho87 and Fgr2 sustain growth well at acidic pH≤6 in high ambient Pi; while a triple mutant hardly transports Pi at pH≥6. Pho84 alone among the studied transporters signals to TORC1; it is also most important for tolerance of fluconazole and amphotericin, though the other 3 transporters also contribute to antifungal agent tolerance. *C. albicans* may be better adapted to Pi acquisition at low pH, a condition rarely encountered during host invasion, but perhaps prevalent in microenvironments shaped by bacterial flora of the GI tract.

234C

Efficacy of Candida albicans-Derived Extracellular Vesicles and fungal b-Glucans in inducing Trained Innate Immune Protection Against Lethal Polymicrobial Sepsis

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Abstract

Background: Fungal-bacterial intra-abdominal infections (IAI) can lead to sepsis with significant morbidity and mortality. We have established a murine model of *Candida albicans* (*Ca*) and *Staphylococcus aureus* (*Sa*) IAI that results in acute lethal sepsis. Prior inoculation with $7x10^6$ low virulence (attenuated) *Candida dubliniensis (Cd)* confers high level protection against lethal polymicrobial IAI. Protection is long-lived and mediated by putative Gr-1^+ myeloid-derived suppressor cells (MDSCs) representing a novel form of trained innate immunity (TII). While induction of TII has potential as a vaccine strategy, the requirement for live *Candida* is limiting. The objective of these studies was to determine a minimum *Cd* inoculum capable of inducing protective TII and explore the potential of fungal extracellular vesicles (EVs) and cell wall components as abiotic alternatives to induce TII. **Methods**: Immunizations included titrated inocula of live *Cd,* or repeated doses of EVs or b-glucan compounds administered i.p. Vaccinated mice were challenged with *Ca/Sa* i.p. 7-14 days later and monitored for survival and sepsis over 10 days. Surviving mice were sacrificed and serum, intraperitoneal lavage fluid and spleens were analyzed for fungal burden and markers of sepsis. **Results**: Live *Cd* vaccination-induced protection was ineffective at inocula $<1x10^6$. Among the b-glucan products, immunization with alkali treated purified b-glucan or d-zymosan showed delayed mortality, lower sepsis scores, and reduced microbial burden. EV vaccination resulted in lower sepsis scores, but no significant effect on mortality. **Conclusion**: These results suggest that immunization with b-glucans represent potential alternatives to live *Cd* for protection against polymicrobial IAI and sepsis.

235A

Trends in pediatric candidemia: 11 years' survey of epidemiology, anti-fungal susceptibility and patient characteristics in a children's hospital

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Abstract

Candida bloodstream infections (CBSIs) have decreased among pediatric populations in the United States, but remain an important cause of morbidity and mortality. Species distributions and susceptibility patterns of CBSI isolates diverge widely between children and adults. Awareness of these patterns can inform clinical decision-making for empiric or pre-emptive therapy of children at risk for candidemia. In this work, CBSIs occurring from 2006-2016 among patients in a large quaternary care children's hospital were analyzed for age specific trends in incidence rate, risk factors for breakthrough-CBSI and death, as well as underlying conditions. Candida species distributions and susceptibility patterns were evaluated in addition to antifungal agent use. We found that the overall incidence rate of CBSI among this complex patient population was 1.97/1,000 patient-days. About half of CBSI episodes occurred in immunocompetent children and 14% in Neonatal Intensive Care Unit (NICU) patients. Candida parapsilosis was the predominant species (35.6%), followed by C. albicans (29.8%). C. lusitaniae (13.4%) was the third most prevalent species isolated surpassing C. glabrata (7.7%). Antifungal resistance was minimal: 96.7% of isolates were fluconazole-, 99% were micafungin-, and all were amphotericin susceptible. Liposomal amphotericin was the most commonly prescribed antifungal agent including for NICU patients. CBSI-associated mortality was 13.7%; no deaths were associated with CBSI among NICU patients after 2011. We conclude that pediatric CBSI characteristics differ substantially from those in adults. Improved management of underlying diseases and antimicrobial stewardship may further decrease morbidity and mortality from CBSI while continuing to maintain low resistance rates among Candida isolates.

236B

One Health approach based on soil sampling to decipher Candida epidemiology in an area of Candida auris transmission

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Abstract

One health is an integrated approach which includes multidisciplinary studies to address a global epidemiologic feature of the disease. In the case of fungal diseases, the literature concerning this concept is scarce. The aim of the study was to characterize the yeast biodiversity in an area of northern region of Colombia where candidiasis due to *Candida auris* has been frequently reported. Considering that the ecological niche of this emerging pathogen is unknown, an ecological and microbiological study was conducted based on the transect methodology and a targeted sampling. The transect crossed the Valledupar surroundings from the north to the south evidencing different biotopes. Soil and hole in tree trunk samples (n=100) were cultured on Can ID medium at 37 °C and all isolated yeasts were identified by MALDI TOFF and ITS1/2 sequencing. Rice crops, orchards, pasture and trees as *Eucalyptus*, *Hevea*, *Ceiba* and *Prosopis sp* allow us to isolated *Candida* yeasts (*C. parapsilosis, C. metapsilosis, C. guillermondii, C. palmioleophila, C. sanyaensis*) but no *C auris*. Furthermore other yeast (*Trichosporon ashaii, Dirkmeia churashimaensis,* Ustiliago sp,…) were recovered, some of them being potentially involved in human cases. More eco-epidemiological study are need to understand the ecologic niche of C auris.

237C

Use of open resources and literature to teach undergraduate students about *Candida* and other yeast.

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Abstract

Many undergraduate schools might not routinely teach a mycology class. If a school does teach a mycology class, students might not want to invest in a textbook and the options for resale are probably much smaller than with a class in which more students routinely enroll. This presentation includes information about open resources and literature that might be included in a section of a mycology or typical microbiology class. Since this meeting is focused on *Candida*, there will be an emphasis on outside resources that can be used to teach about *Candida* and other yeast. These resources are important to show both students and faculty some select information that can be found online. However, it should be noted, that if a teacher wants to expand this concept to teaching an entire mycology class with open resources, there are ample resources available.

There will be introductory resources presenting to enable students to understand some basic features relating to all fungi, including *Candida* and yeast. This topic will be expanded to include specific features relating to *Candida* and yeast. The presentation will include other topics such as immune responses and diseases, cell biology, molecular biology techniques and research and some select literature articles. All of these sources can help faculty teach students about these topics without having students purchase a textbook.

238A

Candida krusei candidemia in North India: A single genotype outbreak in neonatal intensive care units and genetically diverse strains in multiple hospitals detected by microsatellite typing and genome sequencing.

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Abstract

Candida krusei accounts for 2.8% of invasive candidiasis worldwide. Notably, recent reports from the Eastern United States, Latin America and Europe documented posaconazole, voriconazole and amphotericin B resistance respectively in *C. krusei* clinical isolates. *C. krusei* as an agent of candidemia deserves special attention as this yeast is intrinsically resistant to fluconazole, a first line antifungal to treat candidemia in resource limited countries. We analyzed genetic diversity of *C. krusei* candidemia isolates collected from 8 hospitals in North India. A total of 137 isolates predominately from cases of candidemia (76%) and others from candiduria and vaginal candidiasis were identified by MALDI-TOF. AFST was done using CLSI broth microdilution method. Microsatellite (STR) typing using 9 STR markers was done and genome sequencing was used to evaluate the adequacy of microsatellite typing.

Notably, 70% of candidemia cases occurred in neonatal units. Interestingly, a single STR type each was implicated in ongoing candidemia outbreaks in NICUs of two hospitals. Overall, reduced susceptibility of *C. krusei* against fluconazole (GM 18.34mg/L) was observed whereas isavuconazole (0.06mg/L) and voriconazole (0.08mg/L) exhibited highly potent activity followed by posaconazole (0.23 mg/L). Remarkably, 17.5% isolates were resistant to itraconazole. Marked genetic diversity with 49 diverse STR types were circulating in the remaining six hospitals. Whole genome sequencing and STR typing gave concordant results i.e., identical STR types differed by 2 to 6 SNPs and different STR types had 579 core SNPs differences confirming that microsatellite typing is a reliable method to study the genetic diversity in *C. krusei*.

239A

Impact of *Candida auris* colonization on human skin-associated fungal and bacterial communities

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Abstract

Background
Candida auris is an emerging pathogen, with ongoing transmissions reported in North American nursing homes since 2017. *C.auris* persistently colonizes human skin and causes difficult to treat bloodstream infections.

Methods:
To identify risk-factors and primary colonization sites, 51 patients were screened at 10 body-sites over 3 months at a skilled-nursing-center experiencing a C.auris outbreak. 1,200 skin samples were independently cultured and sequenced, generating >60x10^6 high-quality 16S-rRNA and ITS1 sequences. The impact of *C. auris* on fungal communities was assessed by identifying community state types (CSTs) defined by the cluster-size minimizing the gap-statistic on a bootstrapped-partition-around-medoids analysis. To explore temporal variation, the probability of transition between CSTs was modeled using site-specific markov chains. Bacteria covarying with *C. auris* were identified using sparse projection-to-latent-structure analysis (sPLS).

Results:
Fewer than 20% of subjects retained the same site-specific CST over time, indicating large-scale temporal instability. Of the 4 CSTs, CST1 was dominated by typical skin-associated commensals (e.g., *Malassezia restricta*); CST2 by *C. auris;* CST3 by other Candida species; and CST4 by *M. slooffiae*. Although CST1 had the highest average self-transition probability P(CST1|CST1)=0.69, the *C. auris* dominant CST exhibited remarkable site-specific stability at the toeweb P(CST2|CST2)=0.72 with lability at other sites. sPLS revealed *C. auris* to be positively and *M. restricta* negatively-correlated with *Staphylococcus pettnkoferi* and *Corynebacterium massillense*.

Conclusion:
Our data demonstrate the site-specific resilience of *C. auris* once established in the skin microbiome with implications for the control and surveillance of *C. auris* in hospital settings.

240B

Effect of exposition to broad-spectrum antibiotics on the gut mycobiota of healthy volunteers

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Abstract

Background

Antibiotic exposure, including third-generation cephalosporins, impacts the intestinal bacterial microbiota but its effect on the human fungal microbiota (mycobiota) is unknown.

Materials and Methods

Two groups of 11 healthy volunteers received either intravenous ceftriaxone (1 g/24 h) or cefotaxime (1 g/8 h) for 3 days. Fecal samples were collected before treatment at days (D) -15, -7, -1 and daily between D1 and D4, and at D7, D10, D15, D30, D90 and D180 to quantify fungal DNA (qPCR of the 18S rRNA gene) and to profile the fungal internal transcribed spacer (ITS1) rRNA region (HiSeq platform, Illumina).

Fungal DNA concentrations before treatment were compared to D4, D7, D10, D30 and D180. Differential analyses were performed to compare genera ITS1 abundance before treatment with D4, D7, D10, D30 and D180 for each volunteer.

Results

Before exposition to antibiotics, the mycobiota of the volunteers was characterized by a high within and between diversity (Bray-Curtis dissimilarity within/between: median: 0.85/0.88, min: 0.48/0.38, max: 0.98/1.0). Antibiotic treatments significantly increased fungal DNA concentration at D7. Discriminative analysis identified disparately represented fungal taxa in the volunteers' samples before and after antibiotic treatment. Especially, *Penicillium* genus was less abundant for all tested time-points, while *Candida* spp. abundance was increased only at D4.

Conclusion

Clinical regimens of ceftriaxone or cefotaxime impacted significantly the gut mycobiota of healthy individuals. Further analyses will be conducted to assess the effect of the antibiotic treatments on the specific yeasts *Candida albicans* and *Saccharomyces cerevisiae.*

241C

Stable intestinal colonization of laboratory mice with *Candida albicans* without antibiotic treatment

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Abstract

Microbial dysbiosis due to antibiotic treatment is a major risk factor for candidiasis and increasing evidence suggests that different bacteria can limit *Candida* colonization in mice. However, only few distinct bacterial species responsible for colonization resistance have been identified.

We aimed to identify additional bacteria conferring colonization resistance by utilizing natural microbiome variation in laboratory mice. Following microbiome analysis by 16S and ITS sequencing of fecal samples, five out of 18 analyzed C57BL/6 breeding colonies were selected based on differences in α-diversity, β-diversity, and the ratio of Firmicutes to Bacteroidetes. Non-treated mice and animals receiving different antibiotic treatments were inoculated orally with *C. albicans* once, and both bacterial and fungal burden were quantified as colony forming units (CFU) at several time points throughout 14 days.

In contrast to several previous reports that adult laboratory mice are naturally resistant to *C. albicans* colonization, stable colonization with up to 1×10^5 *C. albicans* CFU/g in feces and gut was detected in the absence of antibiotic treatment in mice from all selected breeding colonies. Depletion of intestinal bacteria by antibiotics enhanced colonization in all mice with the highest increase observed following combination therapy.

Despite considerable variation in the microbiome, no significant differences in colonization resistance were observed between mice from different breeding colonies within the same treatment group.

These results confirm that antibiotic-induced dysbiosis enhances colonization with *C. albicans* but surprisingly revealed considerable stable colonization also in the absence of dysbiosis.

242A

The Candida albicans secreted lipase LIP2 reduces IL-17 production by γδ T cells

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Abstract

The commensal fungi, *Candida albicans* colonizes the mammalian gut, reproductive tract, and skin of most healthy humans. Colonization typically occurs during childhood and the initial infecting clone may persist for years or even the lifetime of the host. This is typically asymptomatic, but perturbations of the bacterial microbiota or host damages caused by surgery or immunomodulatory drugs can result in fungal overgrowth, inflammation, and even dissemination from the commensal niche.We hypothesize that *C. albicans* uses specific mechanisms to overcome host immunity and establish stable infections in the gut. We previously discovered transcriptional regulatory circuits that regulate the fitness of *C.albicans* in the mammalian gastrointestinal (GI) tract vs. the bloodstream. To identify non transcription factors with an altered capacity to colonize the mouse gut, we used the **G**ene **R**eplacement **A**nd **C**onditional **E**xpression (GRACE) mutants library. The GRACE library targets 1,790 genes and among them 1,411 are described as non-essential. We tested their fitness in a mouse model of GI commensalism and we identify ~200 non-essential genes required for the colonization. Moreover, we hypothesize that *C. albicans* colonization can affect the host cells pattern. To that aim, we performed **s**ingle-**c**ell RNA sequencing (scRNA-seq) from the small intestine cells, in mock or *C.albicans* infections at different time points. These preliminary results indicate that the epithelial and immune cells changed during infection. All together these results, will participate to demonstrate the host-*C. albicans* interactions in the GI tract.

243B

Study of the interaction between vaginal and intestinal *lactobacilli*, *Candida albicans* and *Candida glabrata*

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Abstract

The success of *Candida glabrata* and *C. albicans* as human commensals/pathogens depends on their ability to cope with the bacterial microflora that has been found to restrain their pathogenic potential. In this work the inhibitory role of *Lactobacillus gasseri* and *L. reuteri*, highly abundant species of the vaginal and intestinal microbiomes, over *C. albicans* and *C. glabrata* was investigated coupling physiological studies with genome-wide scale analyses. The results obtained revealed that while in co-culture *L. gasseri* and *L. reuteri* reduced growth rate and cell viability of the two *Candida* species, however, this could not be attributed to a potential toxic effect exerted by the lactic acid produced by the bacteria. The role of other components accumulated in the culture broth in mediating the observed interference effect will be discussed, including the exopolysaccharide released by the two *lactobacilli* species and that was found to have a strong anti-*Candida* potential, both in planktonic and in biofilm conditions. The presence of *L. gasseri* and *L. reuteri* also attenuated virulence traits of *Candida* cells against epithelial cells and also against the wax *Galleria mellonella*. The role of competition for adhesion sites and immunomodulation in this effect of the *lactobacilli* over pathogenic *Candida* traits will be discussed. The link between these phenotypic traits and the remodelling of the genomic expression of the *Candida* and *lactobacilli* species, including the identification of the molecular players governing the interaction, will also be discussed based on RNA-seq results performed in the co-culture settings.

244C

The interactions between *Candida albicans* and human gut microbiome

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Abstract

The human gut is an anaerobic chamber with limited nutrients and space, and all microorganisms in the gut maintain a relatively stable population with little fluctuation. Because of the constraint of nutrient and space in the gut, *C. albicans* is aware of the presence of its neighboring bacteria. Thereby, *C. albicans* behaves differently in the human gut than it does alone in the test tube. As a stable human gut commensal, *C. albicans* is also able to gain enough resource to maintain its own population in the presence of gut bacteria. How it responds to the presence of bacteria is the topic that we are interested in. We study mechanisms that allow *C. albicans* to sense and respond to the presence of gut bacteria in the in vitro laboratory setup which mimics the gut environment (anaerobic and nutrient-limited). Transcriptome analysis reveals that many genes with unknown function are highly induced in the presence of bacteria. We also examine the response of gut microbiome in the presence of *C. albicans*.

245A

Cross-Kingdom Interactions between *Candida species* and *Enterococcus faecalis* during Commensal Growth

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Abstract

Candida species colonize the infant gut microbiome within the first few days of life. *C. albicans* is the most common fungal colonizer of healthy infants, while in pre-term infants, *C. paraspilosis* is surpassing *C. albicans* as the most prevalent Candida species. Despite the large number of microbiome-related studies, we are just beginning to understand how microbial members of these communities interact. In this study, we begin to look at these interactions by re-creating minimal communities *in vitro*. Using Dual RNAseq, changes in gene expression will be assayed for co-cultures of *C. albicans* or *C. parapsilosis* and *Enterococcus faecalis*, a common gut Firmicute. This simplified system will allow us to begin to understand interspecies interactions in a way that has not been possible with whole microbiome sequencing studies.

247C

Transcriptomic analysis of chemotherapy-associated oropharyngeal candidiasis reveals a role of indigenous bacteria in mucosal cell death.

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Abstract

Cytotoxic chemotherapy is a risk factor for oropharyngeal candidiasis (OPC), acting through myelosuppression and damage to mucosal tissues. Recent work in our 5-Fu chemotherapy mouse model showed that *C. albicans* increases bacterial numbers and dramatically reduces the diversity of the host microbiome. Overgrowth of *Enterococcus sps*., which dominated the oral microbiome, was associated with markedly greater invasion of tongue tissue by *C. albicans* filaments, and this was alleviated by treatment with antibiotics. We hypothesized that interkingdom interactions between host*, C. albicans* and *Enterococcus* could account for these observations. We explored bacterially-induced gene expression changes through RNA-Seq profiling of tongue tissues from mice receiving 5-Fu and *C. albicans*, with and without antibiotics. Among the significantly differentially expressed genes we found 81 genes overexpressed and 117 genes under-expressed in antibiotics-depleted mice. Gene Ontology enrichment analysis for biological processes revealed that genes negatively influencing epithelial apoptosis and positively influencing proliferation were significantly enriched in antibiotics-depleted mice, implicating bacterially-enhanced epithelial apoptosis in mucosal damage and fungal invasion. These results were validated experimentally by showing that enterococcal isolates from mice with oropharyngeal candidiasis secrete hydrogen peroxide and induce oral epithelial cell apoptosis *in vitro*. Importantly, we showed that *C. albicans* promotes H_2O_2 production by these isolates leading to increased oral epithelial cell apoptosis, which was inhibited by the addition of catalase. Our findings point to a new mechanism of pathogenic synergy between *C. albicans* and *E. faecalis* which may be responsible for greater invasion of epithelial tissues by *C. albicans* hyphae.

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