(R-WTM) was significantly higher (P < 0.01) than those with mutation (R-WTM) and the sensitive isolates (1.2-11 vs. 0.2-2.5, and 0.3-2.2 fold, respectively). Although the R-WTM and R-L-W TM background (P < 0.05) CD82 and MDR1 expression compared to S isolates, noticeable variation was not seen among the other genes. Protein-homology modeling and molecular docking revealed that the mutations in the ERG11 gene were responsible for structural alteration and low binding efficiency between ERG11p and ligands. Isolates with ERG11 mutations also possessed AT220C in ERG11 and together T103C-G701A in SPS2.

Conclusions: Non-enzymatic mutations in the ERG11 gene and coordinated-overexpression of various genes including different transporters, epigenetic hypothesises pathway, transcription factors, and stress-response genes are associated with azole resistance in clinical isolates of C. tropicalis.

S5.3a Unraveling the genetic determinants of virulence in Cryptococcus neoformans

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S5.3.1 Cellular phenolmorphism and fungal virulence, September 22, 2022, 3:00 PM - 4:30 PM

Cryptococcus neoformans is a human pathogenic brain-crust yeast that can cause central nervous system (CNS), predominantly in immunocompromised individuals. The patient outcome depends on both host and pathogen-specific factors, including C. neoformans genotypes. A groundbreaking 2012 study was the first to show that patient outcome is associated with genetic differences between C. neoformans isolates. Subpopulation-wide-sequencing studies have revealed over 150 sequence types (ST) of C. neoformans that are associated with both geographic location and clinical outcome. All these studies have been broad, examining the severity of these cranial cryptococcal phenotypes in a collection of highly diverse strains. We chose a narrow focus and collected various genotypic and phenotypic data from a single ST: ST53. ST53 is a common sequence type isolated from patients and is the most common clinical isolate found in the sub-Saharan African country of Uganda. Previously, we performed whole genome sequencing on 38 ST51 Ugandan clinical isolates. We identified 652 unique SNPs in this ST53 population compared to the H99 reference genome. We also showed that ST53 contained two subpopulations: ST51 and ST53B. In the current study, we further characterized the genotypic, phenotypic, and virulence differences between these 38 clinical isolates. Using Illumina sequence data, we identified a pattern of linkage disequilibrium that suggested that ST51A and ST51B are evolving separately. We performed long-read sequencing on each isolate to investigate chromosomal changes and large rearrangement alterations. We were able to identify a chromosomal translocation event within parts of chromosome 11 that had recombined with chromosome 5. Additionally, we characterized several in vitro phenotypes for each isolate and identified these distinct phenotypic clusters based on cell wall challenge and growth experiments. Next, we infected mice with 35 isolates and observed eight distinct disease manifestations, including isolates that caused non-CNS infections. Overall, by working within a single sequence type, we can gain a deeper understanding of how some small genetic changes can impact strain-specific phenotypes while others have no discernible effect. Eventually, these data can be used to provide valuable information about how each clinical strain impacts patient outcomes.

S5.3b Fungal spore: Initiators of colonization and infection

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S5.3.2 Cellular phenolmorphism and fungal virulence, September 22, 2022, 3:00 PM - 4:30 PM

Fungi produce unusual and sexual sporues for reproduction and distribution, which can be both in space and time. Distribution in space occurs, by air movement, but also by water or other vectors such as living organisms. Fumonisins from the Aspergillus genus that belong to the order Eurotiales produce unusual sporues called conidia. Conidia are resistant, spore-containing cells and are able to survive unfavorable conditions such as thermal stress, dehydration, osmotic pressure, oxidative stress, variations in pH, and UV. For example, conidia of the fungus Fusarium oxysporum are isolated worldwide and must be regarded as a contamination risk at any time, no matter how ‘safe’ locally to the location of production, but still may become major making into the highest air layers. There is indirect evidence that spores may be able to travel long distances through the air. For example, hyperparasitic species have been shown to travel over thousands of kilometers from the Sahara Desert to the Caribbean Sea.

Distribution in time is occurring as stress-resistant cells remain dormant at one location for an extended period, during conditions that are more favorable for growth. Some ascospores (sexual spores) are extremely stress-resistant and dormant for very long periods. Other species show extended dormancy in a dry state. As microspores are inherently variable, stress resistance versus dormancy from species to species. For example, conidal heat resistance (H99) of various strains of the fungus Paecilomyces variotii ranged between 5.0 to 27.4 min. The intraspecific variation could have profound consequences on diagnostics, virulence, and antifungal treatment in clinical settings.

To understand the mechanism of conidial germination and fungal spore invasion, the presence of nutrients such as inorganic salts, sugars, and amino acids is required. The swelling phase of conidia is also critical for growth. Swollen conidia direct the growth to one side of the cell to grow in a polarized fashion, which leads to the formation of a germ tube (polarized growth). There is a notable drop in stress resistance during the vegetative and polarized growth and genes expressed during these stages might represent novel targets for fungal selection.

S5.3c Investigating the link between phenolmorphism and virulence in Cryptococcus

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S5.3.3 Cellular phenolmorphism and fungal virulence, September 22, 2022, 3:00 PM - 4:30 PM

Objectives: Fungal pathogens Cryptococcus neoformans and Cryptococcus gattii are responsible for hundreds of thousands of annual deaths in immunocompromised individuals. Considerable phenotypic variation is elicited by strains in response to stressors encountered during host infection, including increased capsule and cell size, the release of seed capsules, and the production of giant (> 15 μm), micro- (< 1 μm), and irregular cells. We aimed to investigate whether the production of these morphological variants is associated with virulence using two sets of strains. The first set is a collection of diverse clinical isolates obtained from HIV/AIDS patients in Benin with accompanying clinical data. The second is a collection of lineages derived from the C. neoformans type strain H99 with high genetic similarity but different levels of virulence. Some lineages in this set possess a mutation in SPS2, which encodes a component of the SAG1 heparin acetylation complex that has previously been implicated in their hypervirulence.

Methods: Isolates were cultured under conditions that simulate stressors encountered in vivo (DME/M, 5% CO2, 37°C) as these are known to elicit capsule production and induce cell wall changes. Cells were counterstained with DAPI, visualized by light microscopy, and phenotypes were scored. For clinical isolates, MLST analysis was performed to determine their alleles. For H99 strains, Califia mello-kolster subset analysis showed highly conserved and divergent profiles, suggesting that isolates are mixed in clinical infection. "Small"/"globular" cells were associated with lower CD4 counts, negatively correlated with neutrophil inflammatory indicators, and positively correlated with intracellular stress indicators, suggesting that they are produced during infections and may promote proliferation and dissemination. Isolates possessing giant cells, microcells, and seed capsules were rare, but strikingly, were associated with patient death.

In the H99 set, strains from HIV/AIDS lineages had larger average capsule size, greater variation in cell size, and increased production of microcells and seed capsules. Deletion of SPS2 in an intermediate virulence lineage substantially increased its production of microcells and released capsules, consistent with a stretch to hypervirulence. SPS2 loss of function mutations were subsequently identified in clinical isolates and were found to be significantly correlated with patient death. Expansion of a TA repeat in the second intron of SPS2 in clinical isolates was positively correlated with cell size and capsule size, suggesting it also affects SPS2 function.

Conclusion: Our results extend the evidence for a link between phenolmorphism and virulence, with a likely role for epigenetic mechanisms mediated by SAG1-induced heparin acetylation.

S5.3d How mitochondrial complex I proteins in Candida albicans moderate phagocytosis and the production of pro-inflammatory cytokines in murine macrophages and dendritic cells

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5PUMC and fungal virulence, September 22, 2022, 3:00 PM - 4:30 PM

Objectives: Inhibition of migration in Candida albicans impairs its colonization in the host tissues and causes candidiasis in a murine vascular candidiasis model. Accordingly, blunting of the mitochondrial electron transport chain (ETC) of C. albicans by respiratory inhibitors promotes phagocytosis by increasing exposure of glucans which could be due to the mannase reduction. In our model, we have reported that 95% mannase reduction in goa1 and a deletion mutant of an ETC Complex I (goa1) results in specifically, oppositely decreased phagocytosis. To understand such a difference, we broaden our investigation with three C2 respiratory mutants, which are either fungal-specific (gaa1 and gaa2) or broadly conserved subunits (ubn3/f1a) for cell wall analysis and immune response measurements.

Methods: We characterized mutant cell wall defects in these mutants, then analyzed their respective survival in macrophages. Fungal internalization into macrophages was revisited under fluorescent microscopy and live-cell imaging and analyzed through flow cytometry analysis. Cytochrome production in dendritic cells (DC) infected by fungal cells was measured by Nanoarray technology and the transcriptional profile of murine macrophages-infected by different mutants was compared.

Results: We found that phosphoglucomutase (PPI) reduction in gaa1 and gaa2 and phospholipase (PLM) reduction in ubn3/f1a correlate with robust inhibition of cytokine. PPI loss in gaa1 and gaa2 fails to promote phagocytosis or enhances spontaneous engulfing. The case of PPI malfunction results from reduced phosphorylation of the Cd4 MAPK in gaa1 and ubn3/f1a. In contrast other three mutants, phagocytosis and cytokine production of ubn3/f1a more resemble WT cells, which have shown an ~30% glucan reduction due to a defective Mek1 MAPK response. The divergent immune responses to these C2 mutants are shown at the transcriptional level in infected macrophages. We found that these well-characterized host receptors such as toll and SRCR24 for PPI, PLM, and glucan ligands are not significantly affected at 1 h post-infection. However, the downstream receptor CDFM, integrin ICAM, and growth factor receptors are downregulated along with a generally downregulated monokine and antigen processing/presentation. In addition, the host metabolic processes, oxidative stress-induced senescence, apoptosis, and signaling pathways for Rgf1/Efr1, the AMPK/CREB, and TLR2 pathway, are each individually affected in the host cells.

Conclusion: We speculate that mitochondrial signals of fungal origin may also be sensed by the host immune cells to coordinate the immune response together with cell wall modifications and metabolic during the early stage of infection.
Cutaneous manifestations of deep fungal infections: A retrospective study from one tertiary hospital

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S5.4 Free oral paper session, September 22, 2022, 3:00 PM - 4:30 PM

Objectives: Analysis of the cutaneous manifestations in patients with deep fungal infections to provide a basis for clinical differentiation and diagnosis.

Methods: Patients who presented to our hospital from 2016 to 2021 were definitively diagnosed with deep fungal infections by histopathology and mycological detection. Isolates of focal infections were cultured in vitro on SDA or MEA media for 14 days and the species were identified by morphological or molecular analysis. Relevant clinical data on epidemiology, skin manifestations, underlying disease, causative fungal agent, treatment, and outcomes are collected and analyzed.

Results: A total of 15 patients were diagnosed with deep fungal infections. The respiratory system (7/15) was the most easily involved primary focus of deep fungal infection, digestive system (3/15) and nervous system (2/15) were less common. The mean age of the patients was 50.30 years. Of these, 8 were males. More than half of the cases (7/15) were presented in immunosuppressed patients, including long-term glucocorticoid use, organ transplantation, tuberculosis infection, and malignancy. Skin manifestations varied, with plaques (5/15) being the most common type of lesion, and then papules (4/15), nodules (2/15), patches (2/15), and ulcers (2/15). Candida spp. (9/15) was the most common pathogens, followed by Talaromyces marneffei (2/15) (Fig. 1a), Cryptococcus spp. (2/15) (Fig. 1b), and Aspergillus spp. (2/15). One case had co-infection with C. albicans and Aspergillus spp.

Conclusions: Patients with deep fungal infections are often accompanied by skin manifestations, which vary between patients with deep fungal infections caused by different pathogenic fungi.