(R-WT) was significantly higher (P < 0.05) than those with mutation (R-WM) and the sensitive isolates (1.2-11 vs. 0.2-5, and 0.3-2.2 fold, respectively). Although the R-WT and R-WM isolates (P < 0.05) DE2 and M38X 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 loss binding efficiency between ERG11p and ligands. Isolates with ERG11 mutations also possessed K220C in ERG11 together with T301C, G371A mutations in CNC2.

Conclusion: Nonhomologous mutations in the ERG11 gene and coordinated overexpression of various genes including different transporters, engineered synthetic pathway, transcription factors, and stress-response genes are associated with azole resistance in clinical isolates of C. neoformans.

55.3a Unravelling the genetic determinants of virulence in Cryptococcus neoformans

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55.3.1 Cellulose phosphorylation and fungal virulence, September 22, 2022, 2:00 PM - 4:30 PM

Cryptococcus neoformans is a human pathogenic basidiomycete yeast that can cause cryptococcosis meningitis (CM), 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-single-nucleotide studies have revealed over 100 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 disease across global populations. In this study, we focused on a smaller set of STs and observed eight different disease manifestations, including isolates that cause 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. These data can be used to provide valuable information about how such clinical isolates impact patient outcomes.

55.3.2b Fungal spores: Initiators of colonization and infection

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55.3.2 Cellulose phosphorylation and fungal virulence, September 22, 2022, 2:00 PM - 4:30 PM

Fungi produce annual and sexual spores for reproduction and distribution, which can be in both space and time. Distribution in space occurs, by air movement, but also by water or other vectors such as litter organisms. Phanerogam fungal spore production generally occurs in spring and summer, when the sporophytic stage is most active. Spore dispersal mechanisms vary, and some fungi use the wind to disperse their spores, while others have specialized structures that aid in the dispersal process. The types of spores produced by different fungal species can vary significantly, and understanding these differences is essential for studying the distribution and colonization of fungal species.

Distribution in time is occurring as stress-tolerant cells remain dormant at one location for an extended period, allowing conditions that are more favorable for growth. Some spores (sexual spores) are extremely stress-tolerant and dormant for very long periods. Other species exhibit dormancy in a dry state. As asexual spores, these spores are inherently variable, stress resistance varies between strains from the same species. For example, conidial heat resistance (KoF1) of various strains of the fungus Phaeococcomyces variabilis ranged between 5.5 to 27.4 min. The intrinsic variation could have profound consequences on diagnostics, virulence, and antifungal treatment in clinical settings.

The discovery of conidial germination as the source of nutrients in various mycotoxins, sugars, and amino acids is required. The swelling phase of conidia is also called germination growth. Swelling conidia direct the growth to one side of the cell to grow in a polarized fashion, which leads to the formation of a growth pole (polarized growth). There is a notable drop in stress resistance during isotropic and polarized growth and genes expressed during these stages might represent novel targets for fungal selection.

55.3c Investigating the link between phosphorylase and virulence in Cryptococcus

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55.3.3 Cellulose phosphorylation 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 phytopathogenic variability is culminated by strains in response to stress encountered during host infection, including increased capsule and cell size, the release of shed capsule, and the production of gray (> 15 mm), micro- (< 1 mm), and irregular cells. We aimed to investigate whether the production of phenotypic variation is associated with virulence using two sets of strains. The first is a collection of diverse clinical isolates obtained from HIV/AIDS patients in Rome with accompanying clinical data. The second is a collection of isolates derived from the C. neoformans type strain H99 with high genetic similarity but differing levels of virulence. Some isolates in this set possess a mutation in SGF29, which encodes a component of the SGK1-related atypical PKC complex that has previously been implicated in their hypervirulence.

Methods: Isolates were cultured under conditions that simulate stress encountered in vivo (DMEM, 5% FCS, 37°C) as these are known to induce capsule production and induce cell wall changes. Cells were commened with RNA, visualized by light microscopy, and phenotypes were scored. For clinical isolates, MLST analysis was performed to determine their strain. For H99 strains, Gallica-mooff and lateral lesion assays, growth curves, and antifungal susceptibility testing was performed to confirm their intrinsic virulence and growth profiles. Serial block face and regular scanning electron microscopy were used to investigate the intramural morphology of the strain, micro, and irregular cells to confirm that they possess attributes of functional cells.

Results: Substantial phosphorylation was seen across both collections. In the clinical strain set, phosphorytubulin variables fall into two groups associated with differing symptoms. The production of large phosphorytubulin was associated with a higher CD4 count and was negatively correlated with intracranial pressure indicators, suggesting that these are induced in early-stage disease. Small phosphorytubulin were associated with lower CD4 counts, negatively correlated with maximal intracranial indicators, and positively correlated with intracranial pressure indicators, suggesting that they are produced later during infection and may promote proliferation and dissemination. Isolates possessing giant, micro, and shed capsule were rare, but strikingly, they were associated with patient death. In the H99 set, strains from hypervirulent lineages had larger average capsule size, greater variation in cell size, and increased production of microcell and shed capsule. Deletion of SGF29 in an intermediate virulence lineage substantially increased its production of microcell and reduced capsule, consistent with a stretch to hypervirulence. SGF29 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 SGF29 in clinical isolates was prominently correlated with cell and capsule size, suggesting it also affects Spg29 function.

Conclusion: Our results extend the evidence for a link between phosphorylation and virulence, with a likely role for egoprotein mechanism mediated by SGK1-related atypical PKC.

55.3d 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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55.3.4 Cellulose phosphorylation and fungal virulence, September 22, 2022, 3:00 PM - 4:30 PM

Objectives: Inhibition of respiration in Candida albicans impairs its colonization in the host tissues and causes reversion in a murine vascular candidiasis model. Accordingly, blockade of the mitochondrial electron transport chain (ETC) of C. albicans by respiratory inhibitors promotes phagocytosis by increasing exposure of glucos which could be due to the mannose reductase. In our model, we have reported that 5% mannose reductase in galA1, a skeleton mutant of an ETC Complex I (EC) regulator, oppositely decreased phagocytosis. To understand such a difference, we broadened our investigations with three C2 respiratory substrate mutants, which are either fungal-specific (nuo1 and nuo2) or broadly conserved substrates (nuo16A) for cell wall analysis and innate immune responses.

Methods: We characterized mutant cell wall defects in these mutants, then analyzed their respective survival in macrophages. Fungal internalization into macrophages was revealed under fluorescent microscopy and low-cell imaging and analyzed through flow cytometry analysis. Cytokine production in dendritic cells (DCs) induced by fungal cells was measured by Luminex technology and the transcription profile of murine macrophages induced by different mutants was compared. Results: We found that phosphatidylglycerol (PGP) reduction in galA1 and nuo16A and phospholipomannan (PLM) reduction in nuo2 correlate with massive induction of cytokine. PGP loss in nuo16A and galA1 fail to promote phagocytosis but promote increased intracellular killing. The case of PGM12A results from reduced phosphorilation of the C4a MAPK in galA1 and nuo16A. In contrast other two mutants, phosphatase and cytokine production of nuo16A more resemble WT cells, which have shown – 30% glucos 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 noted that these well-characterized host receptors such as dcl2 and TLR2 for PGM, PLM, and glucos ligands are not significantly affected at 1 h post-infection. However, the scavenger receptor CD14, integral ICAM, and growth factor receptors are downregulated along with a generally downregulated anticytokines and antigen processing presentation. In addition, the host metabolic process, cascades stress-induced senescence, apoptosis, and signaling pathways such as RAS/RAC1, the AMPK/CREB, and TLR9 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 participation and modulation during the early stage of infection.