S7.1d

Deciphering the global transcriptomic profile of Candida glabrata during biofilm and planktonic growth phase

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Objective: *Candida glabrata* is an emerging threat in ICU settings of hospitals. Being an opportunist pathogen knowledge about its microbial virulence and exact molecular pathogenesis is still not known. Biofilm formation is considered to be a significant mechanism in its pathogenesis but how biofilm growth phase differentially transcribed the gene expression in comparison to planktonic phase is not elucidated. Analysis and evaluation of comparative transcriptomic profiles of *C. glabrata* in biofilm and planktonic growth phase conditions would be a stride towards the understanding its pathogenesis which may help in unrevealing the key genes responsible for biofilm development and its regulation. Next generation sequencing technologies have advanced the sequence-based research with the advantages of high-throughput, high-sensitivity and high-speed and specially RNA-Sequencing is now being widely used for uncovering multiple facets of transcriptome to facilitate the biological applications. In a present study we made an attempt to determine the whole transcriptomic profile of this pathogen by using RNA seq.

Monitoring of biofilm formation and selection of best biofilm forming *Candida glabrata* isolate on the basis of its biofilm biomass and metabolic activity.

Isolation of total RNA from biofilm and planktonic growth phase and its sequencing by Next Generation Sequencing.Mapping, annotation, detection of differentially expressed genes.Gene Ontology and pathways analysis of selected gene associated with biofilm formation.Validation of differentially expressed genes during biofilm and planktonic growth phase

Methods: Biofilm formation was carried out in 96 well microtitre plate and subsequently biofilm biomass assay was performed with crystal violet assay. Further metabolic assay was carried out by XTT or tetrazolium salt reduction assay. Morphology and architecture of biofilm formation was assessed by Scanning electron microscopy (SEM), Confocal laser scanning microscopy (CLSM). RNA was extracted from planktonic as well as from biofilms using the Hi-PurA yeast RNA purification kit. Sequencing of RNA was outsourced from Sci-Genome pvt. ltd. Aligning, mapping and assembly of transcripts over reference transcriptome was carried out with the Tuxedo pipeline tool and differentially expressed genes (DEGs) were detected. Data visualization for DEGs was carried out by using R studio tools. Gene ontology and pathways analysis of selected gene associated with biofilm formation of RNA seq data was performed by using open source tool Fungi-Fun-2. Validation of few selected DEGs was carried out in wet lab by qPCR.

Results: Clinical strain with NCCPF no 100037 showed best biofilm forming ability and was selected for RNA sequencing. Our bioinformatics analysis showed that 2554 genes differentially expressed during biofilm formation, 1318 genes down regulated and similarly 1236 genes up regulated when compared with planktonic growth phase. Overall analysis from regression curves, volcano curves, heat maps, and pathways analysis of DEGs showed that expression of genes responsible for cell wall formation and other synthetic processes of cell gets down regulated in *C. glabrata* and whole metabolic pathways gets altered in biofilm formation.

Conclusion: The quest of searching the novel anti-fungal targets could be achieved for this inherent azole resistant pathogen by further studying the transcriptomic profile in larger picture.

S7.2

Fungal Interactions with Epithelium Trigger Innate Immune Activation

S7.2a

Epithelial activation by Candida species

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Objective: *Candida albicans* causes mucosal and life threatening systemic infections that contribute to high morbidity and mortality. *C. albicans* hyphae damage host tissue by secreting Candidalysin, a peptide toxin that permeabilises epithelial membranes, triggers c-Fos/MKP-1 signalling pathways, and activates epithelial immunity. Analysis of Ece1p amino acid sequences from different *Candida* species has revealed that additional putative Candidalysin toxins are also present in *C. dubliniensis*, *C. tropicalis* and *C. maltosa*. We compared the different Candidalysins for their ability to cause damage, activate c-Fos/MKP-1 signalling and immune responses in epithelial cells *in vitro*. Furthermore, we assessed the importance of the *C. albicans* Candidalysin in mucosal and systemic murine models of infection.

Methods: Activation of epithelial signalling pathways was investigated by Western blotting. Immune induction was determined by quantification of secreted cytokines by Luminex. Damage was quantified by lactate dehydrogenase assay. Pathogenicity of Candidalysin-expressing and non-expressing *C. albicans* strains was assessed *in vivo* using two mucosal models of oropharyngeal candidiasis and vaginitis, and a systemic intravenous model.

Results: *In vitro* studies demonstrate that the Candidalysins from *C. albicans, C. dubliniensis*, *C. tropicalis* and *C. maltosa* are all capable of damaging epithelial cells, activating c-Fos/MKP-1 signalling pathways, and inducing pro-inflammatory cytokine responses, despite differences in amino acid sequence. *In vivo*, only Candidalysin-expressing *C. albicans* strains were able to damage the host or induce pro-inflammatory cytokines and recruitment of neutrophils to the site of infection. Non-expressing *C. albicans* strains were all attenuated in virulence.

Conclusion: We identify the Candidalysins as a conserved family of fungal peptide toxins, provide mechanistic insights into Candidalysin function, and demonstrate a critical role for Candidalysin in mucosal and systemic *C. albicans* infections.

S7.2

Fungal Interactions with Epithelium Trigger Innate Immune Activation

S7.2b

The Immunopathogenesis of Candida Vaginitis

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Considerable attention has been given to understanding the pathogenesis of vulvovaginal candidiasis (VVC) and recurrent VVC (RVVC) over several decades through clinical studies and animal models. Results of numerous studies eventually led to the consensus that susceptibility to VVC/RVVC is not associated with any apparent deficiencies in adaptive immunity, although protective immune mechanisms and the role of innate immunity remained poorly understood. It was not until an innovative clinical live challenge design was conducted in women that a fuller understanding of the natural history of infection/disease was achieved that provided clues to the pathogenesis of VVC. These studies revealed that symptomatic infection is associated with aggressive inflammation concomitant with the recruitment of polymorphonuclear neutrophils (PMNs) into the vaginal lumen. Subsequent studies in the established mouse model demonstrated that infiltrating PMNs were incapable of reducing fungal burden despite an acute inflammatory state. This led to the hypothesis that VVC/RVVC was associated with immunopathology, involving both *Candida* and the host response as drivers of symptomatic disease. Further studies in mice revealed critical components of the immunopathogenic response, including a requirement for the morphological transition of *C. albicans* to hyphae, vaginal epithelial cell pattern recognition receptors (PRRs), and pro-inflammatory mediators. However, mechanistic details surrounding PMN dysfunction at the vaginal mucosa remained elusive. Ultimately, by testing mouse strains resistant or susceptible to experimental chronic VVC, it was determined that heparan sulfate (HS) in the vaginal environment is a competitive ligand for Mac-1 on PMNs, thereby prohibiting PMN binding to *Candida* to initiate killing. Hence, the outcome of symptomatic VVC/RVVC is postulated to be dependent on a *Candida*-vaginal epithelial cell trigger Hence, the outcome of symptomatic VVC/RVVC is postulated to be dependent on a *Candida*-vaginal epithelial cell trigger, and subsequent PMN-initiated immunopathogenic response involving HS-mediated PMN dysfunction, that effectively renders the neutrophils in a state of anergy.

S7.2c

Pneumocystis Activation of Innate Immune Responses by Lung Epithelial Cells

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Pneumocystis pneumonia (PCP) remains a common cause of morbidity and mortality in patients with immune compromise, particularly in those with AIDS. There are an estimated 400,000 cases of PCP worldwide each year, with mortality ranging from 15 to as high as 80% in resource poor settings. In the absence of effective CD4 cell immunity, the innate immune system assumes a more prominent role in mediating organism clearance as well as initiating inflammatory responses to *Pneumocystis*. Of interest, pro-inflammatory responses in the absence of effective Pc organism clearance, as frequently occurs in CD4 deficiency, strongly promote lung injury and respiratory failure during PCP. Prior studies of innate immunity have focused largely on the roles of alveolar macrophages and dendritic cells in mediating innate responses to *Pneumocystis*. Accumulating evidence supports strong activity of Pc cell wall beta-glucans (including beta-1,3 and beta-1.6 glucans) in potently initiating the release of proinflammatory cytokines including TNF-alpha, IL-6 and MIP-2 during infection. Our data further indicate that alveolar epithelial cells (AECs) provide substantial host recognition and innate inflammatory responses to this fungal organism. For instance, we have shown that AECs generate markedly greater MIP-2 chemokine responses compared to alveolar macrophages on a cell-by-cell basis. Hence, the AECs represent a significant innate immune sensing and response source in the lower respiratory tract during PCP. Additional studies demonstrate that the C-Lectin Receptor (CLR) Family of receptors particularly Decin-1 and Mincle working through the common signaling molecule CARD9 plays a central role in innate immune response to Pneumocystis. Specifically, CD4 depleted mice deficient in CARD9 demonstrated marked impairment of Pc clearance, but also exhibit a dramatic reduction in lung inflammation, indicating important roles for the CLR family in innate immune control of infection and in regulation of host inflammatory responses during infection. While resting AECs do not demonstrate significant Dectin-1 or Mincle, these CLRs are inducted during activation with Pneumocystis and Pc cell wall components. In addition, host cell surface lactosylceramide also participates in AEC interactions and activation in response to the *Pneumocystis* cell wall. Taken together, our studies indicate that interactions of alveolar epithelial cells with *Pneumocystis* cell wall components represents a significant mechanism of host innate immune response during *Pneumocystis* pneumonia.

S7.2d

Differential gene expression of Aspergillus fumigatus and Aspergillus niger interacting with epithelial lung cells

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Objective: *Aspergillus fumigatus* is the main causative agent of aspergillosis. Most infections occur in immunocompromised individuals, indicating an efficient clearance of conidia by the pulmonary defence system in immunocompetent individuals. Infections by other aspergilli like *Aspergillus niger* can occur, but to lesser extent. Previous studies showed that *A. fumigatus* and *A. niger* behave differently in the presence of type II alveolar A549 epithelial cells. *A. fumigatus* is more efficiently internalized by the A549 cells and shows a delay in germination, when compared to *A. niger*. The hyphae of *A. fumigatus*, that escaped the epithelial cells grow parallel to the epithelium, while the *A. niger* hyphae grow away from the epithelial cell layer. This study focusses on the gene expression of *A. fumigatus* and *A. niger* after co-cultivation with A549 cells. Our hypothesis is that the difference in lifestyle between the two aspergilli is also observed in the gene expression profiles.

Methods: RNA of the co-cultivation of the A549 cells with *A. fumigatus* or *A. niger* was isolated and sequenced. The obtained RNA sequences were analysed with custom R and python scripts to obtain the differentially expressed genes and GO terms.

Results: The obtained RNA sequences show big differences in the global gene expression of *A. fumigatus* and *A. niger* upon contact with A549 cells. A total of 545 and 473 genes for respectively *A. fumigatus* and *A. niger* were differently expressed when compared to growth in absence of A549 cells. Of these genes only 53 (~10%) were shared between both species. The different response was also illustrated by the fact that only 4 GO terms were shared between the differentially expressed genes of both gene sets. Genes described in hypoxia regulation and heat shock were found up-regulated in *A. fumigatus* and their homologs in *A. niger*. The *A. fumigatus* thioredoxin reductase and allergen genes were found up-regulated in this fungus, but homologous genes were down-regulated in *A. niger*. After co-cultivation with *A. fumigatus* 62 genes were up and 47 genes were down-regulated in the A549 cells. Co-cultivation with *A. niger* resulted in 17 up and 34 down-regulated genes. GO term related with the immune response were down-regulated in the A549 cells upon exposure to *A. fumigatus*, but not in the case of *A. niger*. This is a strong indication that *A. fumigatus* reprograms the A549 cells to be immunologically less alert.

Conclusion: Our dual transcriptome analysis supports earlier observations of a markedly difference in life style between *A. fumigatus* and *A. niger* when grown in presence of type II epithelial lung cells. These results show an important difference in gene expression, amongst others the downregulation of immune response genes in epithelial cells by *A. fumigatus* and not by *A. niger*.

S7.3

The Real Pathogens: Ajellomycetaceae

S7.3a

Emerging Emergomyces africanus in Africa

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Emergomycosis is caused by several fungal species within a newly-described genus, *Emergomyces*. Evidence of the earliest case of this mycosis (formerly disseminated emmonsiosis) dates back to at least 1992. The recent recognition of these fungi and the disease they cause is attributed to the contemporary use of molecular identification techniques in clinical and research laboratories rather than their sudden emergence as human pathogens. The genus *Emergomyces* is currently placed within the family Ajellomycetaceae alongside other thermally-dimorphic fungal pathogens such as *Histoplasma, Blastomyces*, and *Paracoccidioides*. The largest described burden of emergomycosis is among persons with advanced HIV disease in South Africa, where most cases are attributed to *Es. africanus*. In fact, emergomycosis is the most commonly-diagnosed endemic mycosis in South Africa. Cases have been diagnosed in six of nine South African provinces, which include (in decreasing frequency) Western Cape, Eastern Cape, Gauteng, Free State, Mpumalanga and KwaZulu-Natal provinces. Emergomycosis has also been reported in a patient from Lesotho. Molecular detection of *Es. africanus* was demonstrated in 30% of soils sampled from South Africa (mostly from the Western Cape), including from a wide range of soil habitats. However, attempts to culture *Es. africanus* from soil have thus far been unsuccessful. To date, animal infection with *Emergomyces* has not been demonstrated. Emergomycosis is typically a disseminated disease of immunocompromised hosts. The most common clinical manifestation – best described for disease caused by *Es. africanus* - is the appearance of widespread cutaneous lesions, which can include papules, plaques, or ulcerations. There are no commercially available serological or molecular assays developed specifically for emergomycosis. However, an antigen assay developed for *Histoplasma capsulatum* partially cross-reacts with *Es. africanus*. Diagnosis of emergomycosis is currently made by detection of the yeast phase from affected tissue during histopathology examination or by isolation of the fungus from appropriate specimens such as skin tissue, blood, bone marrow, respiratory tissue, liver tissue and lymph node tissue. Molecular tools can be used to detect *Es. africanus* in clinical and environmental samples and for the identification of clinical isolates to species level. While relatively rare, *Es. africanus* causes a potentially-fatal disseminated mycosis among immunocompromised persons in southern Africa, the only described endemic area for this fungus. Much work remains to be done to understand the full geographic range, ecology, epidemiology and immunopathogenesis of this fungal disease, to understand the full clinical spectrum of disease and to optimise clinical diagnostic and treatment pathways in areas of endemicity.