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An overexpression screen identifies Caa25 as a novel cellular morphogenesis regulator in the human fungal pathogen Candida albicans

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Poster session 3, September 23, 2022, 12:30 PM - 1:30 PM

Objective: Morphological plasticity is one of the key attributes of microbial pathogens contributing to the successful establishment of infection in host tissues. Candida albicans, an opportunistic human fungal pathogen, lives as a commensal in the gut, skin, and gastrointestine of the most healthy individuals. The budding yeast form helps it to disseminate easily in the host system, and the filamentous form (hypha and pseudo-hypha) is believed to invade the host tissue. Strikingly, alterations of gene expression that block cell cycle progression at different stages additionally lead to aberrant cellular morphology in C. albicans. While various morphological states of C. albicans have been well-studied, the search for key players bringing about these changes is far from complete. This is supported by the fact that ~75% of the C. albicans promoters remain functionally uncharacterized. Thus, the primary objective of our study was to identify novel regulators contributing to cellular morphogenesis in C. albicans.

Method: In our current study, we screened an overexpression library of C. albicans ORFeome generated to identify novel regulators contributing to chromosone stability (CST) in C. albicans. The screen involved overexpression of each gene using a tetracycline-inducible promoter for a duration of 12 h, followed by microscopy-based observations to identify associated aberrant cellular morphologies.

Results: Screening of overexpression library of the C. albicans ORFeome identified 14 unique Candidate genes from 1189 genes screened. While the functions of half of these have been notified in C. albicans, the remaining seven genes are not functionally characterized. Each of the seven uncharacterized genes was predicted to be non-essential for viability in C. albicans. Bioinformatic analysis predicts one of these proteins, Caa25, to be carrying a putative antionyme-specific kinases-homolog protein Nkh-10-like DNA-binding domain at its N terminus spanning over a region of 273 amino acids. Sub-cellular localization indicates this protein to be present throughout the nucleus at all stages of the cell cycle. Strikingly, overexpression of this protein led to yeast cells forming chains connected by septa, as visualized by cellulose staining, without hampering nuclear segregation. In addition, a large proportion of cells overexpressing Caa25 were unable to exhibit hyphal morphology when subjected to hyphae-inducing conditions.

Conclusion: In conclusion, our study identified Caa25 as a novel morphogenesis regulator involved in the negative regulation of yeast-hyphal transition in C. albicans. Further studies based on host-pathogen interaction will identify the critical role of Caa25 in the pathobiology of C. albicans and its survival in host-specific niches.

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A tale of fungal burden in chronic suppurative otitis media (CSOM) patients of a tertiary care center of Nepal

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Poster session 2, September 23, 2022, 12:30 PM - 1:30 PM

Objective: The study was designed to find out the fungal etiologies agents in chronic suppurative otitis media (CSOM) patients attending the tertiary care center of Nepal.

Methods: The laboratory-based study was performed at the Department of Clinical Microbiology. Specimens were collected in the ENT and Head and Neck Surgery Department of Tribhuvan University Teaching Hospital (TUTH), Nepal from February 2016 to July 2016. All clinical specimens were collected from hospitalised as well as outdoor patients having CSOM. Specimens were processed according to standard methodology. A total of 117 patients having CSOM were confirmed cases by the stereomicroscopes and their 123 specimens were included in the study. Ear discharge was collected using sterile swab sticks which were labelled and sent to the laboratory for potassium hydroxide (KOH) smear and fungal culture studies.

Results: A total of 125 specimens were collected and processed. Distribution of patients according to the site among the total patients (n = 117), 69 (59.0%) were specimens from the left ear, 42 (35.9%) right ear, and 6 (5.1%) from both ears (bilateral) (Table 1). The 10-30 years age group was highest (34.1%) and followed by 31-50 years having 21.9%. Occupationally, students were higher in number (29.5%) and it was followed by housewives (27.4%). A total of 47.8% of cases were from Kathmandu and remain from different regions of Nepal. Out of 123 specimens, 23 (18.7%) were found KOH mount positive (Table 2). The distribution of fungal isolates is as follows—among total isolates Aspergillus fumigatus 7, A. fumigatus 6, Acremonium 5, Candida albicans 2, Penicillium 2, A. niger 1, C. krusei 1, C. tropicalis 1, Cryptococcus 1, Fusarium 1, and Syncephalastrum racemosum 1 (Table 3).

Conclusion: The prevalence of fungi in CSOM patients was quite high (21.9%). This observation was different from the study of India conducted by Kumar et al. (2017) and in contrast with another researcher in Singapore, Loy et al. (2017). Adams et al. from Pakistan study revealed only 2.1% and Khwakhkh et al. study from Nepal estimated about 1.94% of the Nepalese population suffer from a serious fungal infection annually (commonly in HIV/AIDS and immunocompromised host) which are debilitated by our findings. The possible reason may be due to location, temperature, negligence on mycological complications, and their treatment in Nepal. Treatment of CSOM should be based on the result of fungal culture. CSOM cases are found in all age groups (2-80 years) with various health statuses; different occupations, and in dispersed regions of Nepal. Phenotyping identification is cumbersome and have risk of infection which increases the chance of applying genotyping technique will be beneficial. Antifungal susceptibility testing should be mandatory since it helps in improving clinical outcomes by optimization of antifungal practices. Many CSOM patients complained that they were not cured even long time of use of antibacterial drugs. It clear that fungal etiologic agents can’t be neglected. If I am not wrong, Nepal has no separate designated mycology laboratory. There is also a lack of finding for clinical fungal studies and their awareness regarding fungal pathogens.
### Table 1. Distribution of patients according to site (n=117)

| Site     | Number | Percentage |
|----------|--------|------------|
| Bilateral| 6      | 5.1        |
| Left     | 69     | 59.0       |
| Right    | 42     | 35.9       |
| Total    | 117    | 100.0      |

### Table 2. Distribution of KOH mount results (n=123)

| KOH Mount | Number | Percentage |
|-----------|--------|------------|
| Negative  | 100    | 81.3       |
| Positive  | 23     | 18.7       |
| Total     | 123    | 100.0      |

### Table 3. Distribution of fungal isolates (n=27)

| Fungi                | Number | Percentage |
|----------------------|--------|------------|
| *Aspergillus flavus*  | 7      | 25.9       |
| *Aspergillus fumigatus* | 6    | 22.2       |
| *Acremonium spp.*     | 3      | 11.2       |
| *Candida albicans*    | 2      | 7.4        |
| *Penicillium spp.*    | 2      | 7.4        |
| *Aspergillus niger*   | 1      | 3.7        |
| *Candida krusei*      | 1      | 3.7        |
| *Candida tropicalis*  | 1      | 3.7        |
| *Curvularia spp.*     | 1      | 3.7        |
| *Fusarium oxysporum*  | 1      | 3.7        |
| *Mucor spp.*          | 1      | 3.7        |
| *Syncephalastrum racemosum* | 1 | 3.7 |
| Total                | 27     | 100.0      |
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A screen to identify the regulators of genome stability in the human commensal Candida albicans

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Poster session 3, September 23, 2022, 12:30 PM – 1:10 PM

Objectives: Cell division is a well-regulated process ensuring high fidelity propagation of genetic material to maintain genome stability. A plethora of proteins in distinct cellular pathways, like DNA replication, repair, and segregation contribute to a stable genome. Defects in either of these processes are sensed by cellular surveillance mechanisms ensuring faithful segregation of duplicated DNA during cell division. Failure to correct these defects leads to aneuploidy and rearrangements which may affect the cell stability. On the other hand, rearrangements in the genome are a well-known mechanism for acquiring drug resistance in fungal pathogens including the human commensal Candida albicans. With a major proportion of the genome being underregulated in C. albicans, the regulation of genome stability and antimicrobial resistance, we aimed to identify and characterize novel genome stability regulators in C. albicans using an overexpression screen.

Methods: We utilized an overexpression library of C. albicans genes cloned under the regulatable Tet-O-N promoter. Each construct was stably integrated at the RPS1 locus in a C. albicans chromosomal strain (CSA) reporter strain. The CSA reporter strain contains two fluorescent reporter markers integrated at the same allelic loci of two homologs of chromosome 6.

Chicks and Chicks. The resulting library was used to measure increased genome instability using flow cytometry-based analyses upon expression of individual ORFs. Genome instability was scored by measuring the frequency of loss of one of the fluorescent markers. The distribution between chromosomal loss events and non-chromosomal loss events was made using a third fluorescence market present at the opposite arm of chromosome 6.

Results: Out of the 52,274 ORFs screened, the gene overexpression exhibited an increased genome instability. Two of these genes increased genome instability primarily by chromosome loss, while the remaining three exhibit genome instability due to non-chromosomal loss events. We identified one phylogenetically restricted gene, CSA11, present only in the C. TG-1 clone species of Ascomycota, with a previously unknown function in genome stability. CSA11 is important for cell cycle progression. Overexpression of CSA11 significantly increased the rate of chromosomal chromosome segregation leading to aneuploidy.

Discussion: We identified a phylogenetically restricted gene, CSA11, whose overexpression resulted in chromosome mis-segregation leading to aneuploidy. Further characterization and understanding of the regulatory mechanisms of these Candida genes may reveal unknown pathways for maintaining genome stability and drug resistance. These genes may also serve as novel targets for developing antifungals.

Source:

Joshi P, Legrand M, Das A, Paul T, Cheval M et al, A phylogenetically restricted essential cell cycle progression factor in the human pathogen Candida albicans. 2021, bioRxiv: 2021.2021.04.144188

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Evaluation of the efficacy of fumigation practices on the mycological flora in the Orthopaedic Operation theatre environment at a tertiary care hospital

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Poster session 3, September 23, 2022, 12:30 PM – 1:10 PM

Objectives: To determine the pre and post-fumigation prevalence of fungi in the Orthopaedic Operation Theatre of a tertiary care hospital and characterization of fungal isolates.

Method: This is a prospective environmental, analytical study conducted from July 2021 to January 2022. Pre- and post-fumigation samples were taken from Ultra and Modular OF of Orthopaedic department every week by generic methods of air sampling using Turvy samoution method (1051 series) on SSA and also by surface sampling using swabs. The fungal load of air was measured by calculating the number of CFU per cubic meter (CFU/m3) of air by Onokosorla formula. Fumigation of OF was done with a complex formulation of stabilized hydrogen peroxide 11% w/v with other natural solutions 0.01% (Racahisalit),

All surface samples were inoculated on SSA with chloramphenicol and all plates were incubated at 22° and 37°C. The isolates were identified by using standard mycological procedures. Statistical analysis was done using a T-test.

Results: Out of 19 surface sampling, fumigation was found to be 100% effective only on 3 occasions (15.78%) in Ultra OF and on 7 occasions (36.84%) in Modular OF. In air sampling ≥5% reduction was found in only 4 samplings (21.05%) in Ultra OF and 10 samplings (52.63%) in Modular OF. The counts were much more than the WHO permissible limits. A total of 14 species of fungi were isolated belonging to 11 genera.

The most common isolate was Aspergillus flavus, followed by smoke iodophorophenyl, A. flavus, Gladiolus spp, Casarea spp., Bipolaris spicigea, A. fumigatus, etc. in both Ultra and Modular OF.

Additionally, Exophiala spp and Rospomaceae spp were isolated in Ultra OF.

The concentrations of fungi in Ultra OF before and after fumigation were the range 22.11-58.97 CFU/m3 and 7.53-31.19 CFU/m3, respectively. Whereas, in Modular OF, the range was 14.74-56.86 CFU/m3 and 7.37-29.48 CFU/m3, respectively. Percentage reduction of fungi following fumigation with Racahisalit varied from 0% to 75% both in Ultra OF and Modular OF. The statistically correlated P-value from pre- and post-fumigation concentrations in Ultra and Modular OF were found to be 0.002 and 0.002 respectively which was found significant by Ttest, albeit ineffective as per standards.

Conclusions: In accordance with our findings, Racachisalit has been reported to be less effective even by other manuscripts. Hence, this needs to be replaced by more effective fumigants. The ineffectiveness of fumigation in Ultra OF is most probably due to the lack of HEPA filters and not strictly following up of aseptic protocols. Modular OF ineffective maintenance of MA and lack of periodic cleaning up of HEPA filters may be contributing factors.

Hence, implementing more stringent, frequent, and comprehensive disinfection and cleaning procedures, educating and motivating the health care personnel can help to improve the air quality of OFs that may aid in reducing post-operatives infections.

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