Discovering the transcriptional network controlling flocculation in Candida albicans

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Abstract

Candida albicans is a commensal of the human microbiota and is also the most common human fungal pathogen. Changes to the host immune system, pH, and resident microbiota can lead to C. albicans overgrowth, causing life-threatening disseminated infections. Caspofungin acetate is frequently used to treat invasive Candida infections in the clinic. Interestingly, C. albicans yeast cells flocculate, i.e. they tightly adhere to one another, in response to caspofungin treatment. These adhered cells form structures akin to biofilms, surface attached microbial communities surrounded by extracellular matrices. We identified nine transcription factors (TFs) controlling caspofungin-induced flocculation in C. albicans, several of which are known to also regulate biofilm development. We hypothesize that genes regulated by these TFs may be functionally relevant to both flocculation and biofilm development. We compared our caspofungin treated and non-treated wildtype C. albicans cells using RNA sequencing and identified twenty-five highly upregulated genes that we hypothesize are involved in 1) cellular responses to caspofungin 2) flocculation, and/or 3) biofilm development. Using CRISPR/Cas9 genome editing, we will delete each of these twenty-five genes that we will test for alterations in flocculation and biofilm development. In addition, using Gene Ontology analysis, we will identify which categories of biological functions our genes of interest are most associated with. Together, this work will illuminate the regulatory mechanisms and downstream effectors contributing to flocculation and may identify novel mediators of biofilm development and caspofungin resistance or tolerance.

Objectives

- Use DEseq2 and Gene Ontology analysis on RNA sequencing data of the wildtype in the presence and absence of caspofungin to identify significantly upregulated genes and their putative functions
- Use CRISPR-Cas9 genome editing to delete genes identified from RNA-sequencing analysis
- Screen deletion mutant strains flocculation, biofilm development, and caspofungin sensitivity

Material and Methods

- The transcription factor mutant library as previously described(4)
- Flocculation assays were performed as previously described(2)
- CRISPR-Cas9 deletion mutants were created using a previously described protocol(5)
- Heatmap was created using Bioconductor package on RStudio (6)
- DESeq2 was used for RNA sequencing analysis (6)
- Biofilm assays were performed as previously described (4)

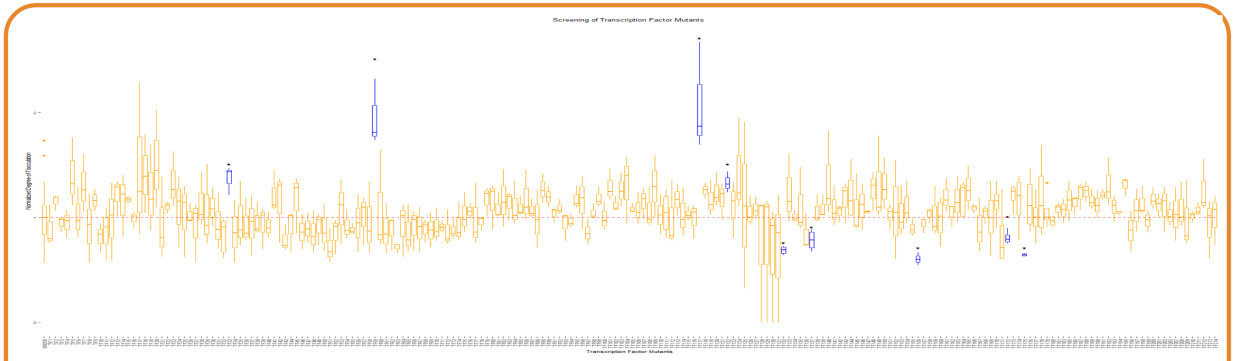

Figure 1- Screening of the transcription factor mutant library identified TF#s 33, 59, 117, 122, 132, 137, 156, 172, and 175, and as mutants with aberrant flocculation.
The nine transcription factors identified were discovered by measuring the degree of flocculation (R value). Mutants with a statistically significant ($p < 0.05$) are colored in blue. The red line signifies the normalized R value of the wildtype for comparison to TF mutants.

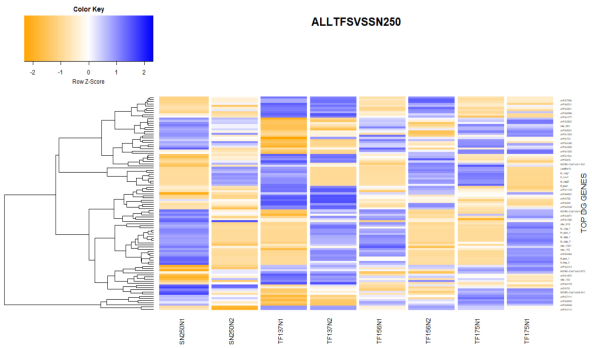

Figure 2a- Heatmap comparing caspofungin treated wildtype and transcription factors of interest RNA sequencing data via RStudio Bioconductor Package.
Our RNA sequencing data for our SN250 treated vs non-treated was compared using Bioconductor to identify genes of interest. Upregulated genes(blue) had a Z-score higher than 0. Downregulated genes had a Z-score lower than 0.

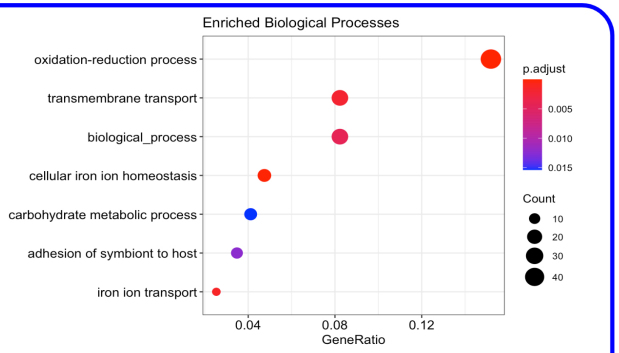

Figure 3b- Gene Ontology (GO) analysis of the RNA-seq data identifies enriched biological processes involved in the caspofungin response and flocculation.
DESeq2 and subsequent GO Term analysis was performed to identify biological processes that are significantly enriched. The GeneRatio represents the percentage of genes within the dataset that belong to a given GO Term with the size of each dot representing the relative count. Seven biological processes were significantly enriched when comparing the wildtype treated with and without Caspofungin (p adj. < 0.05). Genes involved in adhesion are significantly enriched and are likely to play a role in Caspofungin-induced flocculation. Interestingly, oxidation-reduction, transmembrane transport, and iron related processes are also significantly enriched.

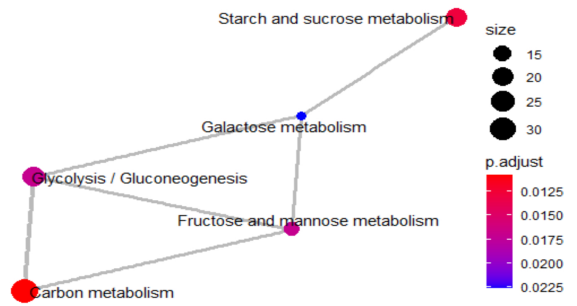

Figure 2C- KEGG Pathway
GSE KEGG pathway analysis revealed a metabolic link between flocculation and sugar dependency.

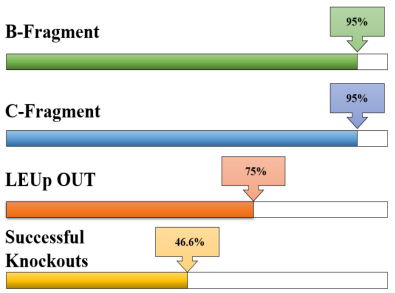

Figure 3- Transformation Progress
Current Progress for each step of the transformation processes. Currently we have 12 genes successfully knocked out for our mutant library. Flocculation and Biofilm analysis will begin once all knockouts are obtained.

Conclusion

- RNA-seq of the wildtype and TF deletion mutants identified genes and processes in C. albicans that are important for flocculation, biofilm development, and caspofungin response.
- Screening a mutant library of twenty-five upregulated genes identified X involved in flocculation and X involved in biofilm development. Additionally, we observed X genes with sensitive phenotypes to caspofungin.
- ALS1, a cell surface adhesion protein, is previously described as a critical adhesion for biofilm development and flocculation. We identified CFL11, a ferric reductase, as a novel mediator of flocculation. Interestingly, a cfl11□□□□is capable of forming biofilms in vivo, however it is defective in in vitro rat catheter models.
- Go Term Kegg Pathway analysis suggest sugar metabolism to be important in response to caspofungin treatment.

Future Directions

- Screen a knockout kinase library to identify kinases upstream of the transcription factors required for flocculation
- Conduct functional analysis to characterize our genes of interest
- Reconstitute genes of interest back into native loci to confirm observed phenotypes was caused by deletion of the gene of interest
- Co-culture flocculation defective mutants with the the wildtype to see if this restores flocculation
- Test for the capability to C. albicans to flocculate in the presence of different sugar sources identified from enriched KEGG pathways

References

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