Probing the role of the Epa1p tandem repeat region in *Candida glabrata* adhesion

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Abstract

Systemic infection by opportunistic pathogens of the *Candida* genus, or invasive candidiasis (IC), has a particularly deadly effect on immunocompromised patients. Invasive candidiasis by *Candida glabrata*, the second most common source of such infections in the US, is dependent upon a variety of cell surface proteins in the adhesin superfamily that promote binding to host tissue and the formation of a biofilm, essential for fungal propagation. Many of these proteins include linker regions containing repetitive amino acid sequences that our lab has shown to exhibit a great deal of variability between isolates from clinical sources. We screened a number of clinical isolates for repeat length variation in a panel of adhesion genes and found that they exhibit a range of repeat copy numbers. To study the contribution of variation in one particular adhesin, Epithelial Adhesin 1 (Epa1p), we heterologously expressed Epa1p in the related yeast, *S. cerevisiae,* and measured the ability of these transgenic yeast to bind to human epithelial cells in a biotic adhesion assay and their levels of Epa1p surface display via fluorescent flow cytometry. We observe that Epa1p with various tandem repeat copy numbers (0, 3, 4, 5 repeats) all confer similar levels of yeast binding to epithelial cells in our current biotic adhesion assay. Through flow cytometry, Epa1p variants with a greater tandem repeat copy number were seen to exhibit different surface display properties than those with fewer copies of the 40 amino acid repeat leading us to conclude that there is an unidentified mechanism through which Epa1p surface display is mediated in our current system.

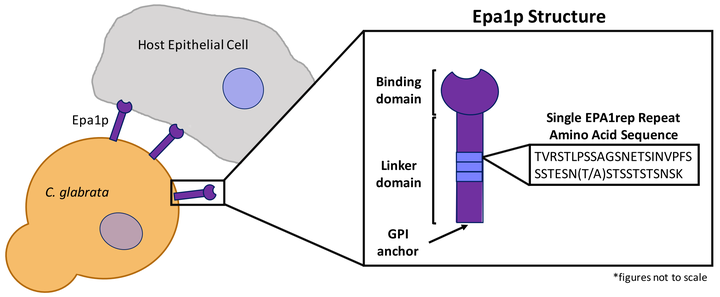
Introduction

Candida glabrata

C. glabrata is an invasive fungal pathogen, closely related to the widely studied yeast, *S. cerevisiae*. It is the second most common cause of IC in the United States, and especially deadly due to its high rates of antibiotic resistance.[1,2] IC by *C. glabrata* relies on a large family of adhesin proteins on the cell surface to bind to host tissue and construct biofilms, essential for parasite propagation.[3]

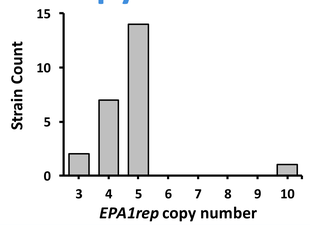
Epithelial adhesin 1

Epa1p is the primary adhesin responsible for binding of *C. glabrata* to host epithelial cells.[4] Epa1p is C-terminally linked to the cell wall via a GPI anchor and binds to cells via the Epa1A lectin-like binding domain capable of binding terminal galactose β1-3 glucose residues on host epithelium.[4,5] Epa1A and the C-terminal GPI are linked with a S/T rich Epa1B domain that includes a tandem repeat region of a 40 amino acid sequence (Epa1rep).[4]



EPA1 is repetitive and variable in copy number

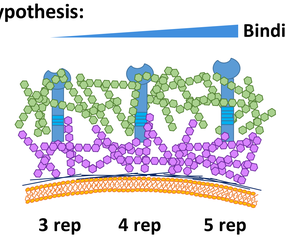
EPA1 codes for a range of copy number variants in the Epa1rep region. Polymerase chain reaction (PCR) and DNA sequencing were used to determine *EPA1rep* Repeat copy number from 24 clinical isolates from the Tufts Medical Center. In the clinical population between three and five tandem copies of the 120 base pair (coding for the 40 amino acid Epa1rep) repeat are seen, while the BG14 reference strain has three repeats.



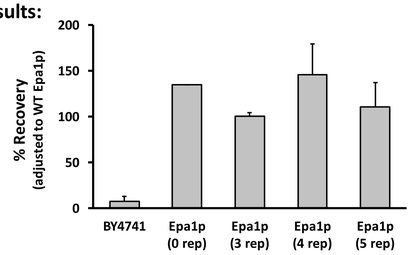
Epa1p repeat copy number confers no significant impact on biotic binding by *S. cerevisiae*

Epa1p variants seen in the clinical population or constructed via QuikChange mutagenesis were expressed heterogeneously in BY4741 *S. cerevisiae* under constitutive expression and their capacities to mediate binding to HeLa epithelial cells were tested.

Hypothesis:

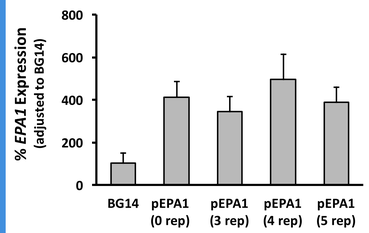


Results:



Previous studies have hypothesized that constructs with reduced linker domain size cannot clear the cell wall and confer poorer binding to cells.[6] We therefore hypothesized that cells expressing Epa1p with higher Epa1rep copy number would exhibit better binding to cells. However, in the biotic adherence assay, all variants bound to epithelial cells with similar strength.

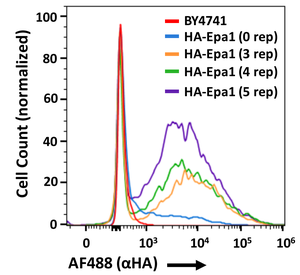
High levels of *EPA1* expression in transgenic strains suggest surface saturation by Epa1p

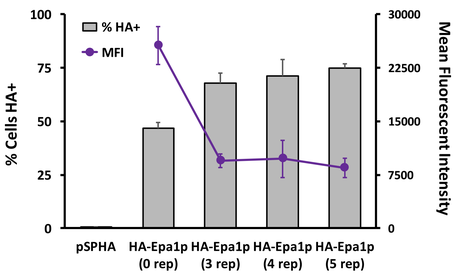


EPA1 mRNA expression assessed through Reverse Transcriptase and quantitative PCR of strains heterogeneously expressing *EPA1* (pEPA1) reveal levels of *EPA1* expression 6-fold higher than BG14 *C. glabrata*. We hypothesize that elevated Epa1p levels saturate the cell surface masking more nuanced effects of variable repeat length on the biotic adherence mediated by Epa1p. All variants of *EPA1* were expressed at similar levels.

HA-Epa1p surface expression varies with repeat copy number

Epa1p variants conjugated to an HA (hemagglutinin) tag (HA-Epa1p) were expressed in BY4741, and their surface displayed was measured through antibody staining and flow cytometry. Cells expressing HA-Epa1p variants with greater repeat copy numbers showed higher proportions of cells positive for HA staining (% HA+), but decreased per-cell intensity (MFI).





Future Directions

- Clone and express *EPA1* copy number variants in *EPA1* knockout *C. glarata* to determine if effects of copy number variation exist in the native system.
- Examine the effects of the longer 10 repeat variant in *S. cerevisiae*.
- Repeat experiments with *EPA1* expressed under a weaker promotor to better study the effect of repeat copy number variation on biotic adherence.
- Determine if variable surface display of Epa1p copy number variants is due to differential post-translational processing or cell wall retention.

References

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