Studying the role of CgMIP1 in the evolution of *Candida glabrata* during adaption to the human host

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Introduction

Candida glabrata is the second most prevalent cause of candidemia, mainly due to its intrinsic and rapidly acquired resistance to azole antifungals. Phylogenetically, *C. glabrata* is more closely related to the baker's yeast *Saccharomyces cerevisiae* than to the most common *Candida* species, *C. albicans*. Recent evolutionary studies have shown that the gene CgMIP1 may have been under high selective pressure during the evolution of *C. glabrata* as a human pathogen. The ortholog MIP1 in *S. cerevisiae* encodes a mitochondrial DNA polymerase.

Defects in mitochondrial functions cause respiratory deficiency and lead to a *petite* phenotype in *C. glabrata* and *S. cerevisiae*. This is linked to increased resistance against azoles, such as fluconazole (FL), due to the overexpression of the transcriptional factor Pdr1. In addition, *petite* clinical isolates of *C. glabrata* have been reported, but data on their virulence is controversial. We study whether the rapidly evolved gene CgMIP1 is involved in the adaptation to the human host and pathogenicity of *C. glabrata*.

Phenotypic characterization of *mip1Δ* from *C. glabrata* and *S. cerevisiae*

Loss of mitochondrial function

Increased resistance to ER stress and fluconazole

PDR1 is involved in ER stress resistance and fluconazole

- *mip1Δ* strains show small colonies and loss of mitochondrial function (white colonies), typical of *petite* mutants phenotype.

- *mip1Δ* strains show, as expected, decreased susceptibility to fluconazole. Contrary to *Scmip1Δ*, *Cgmip1Δ* shows steady growth on ER stress.

- The deletion of *PDR1* triggers a growth defect under stress, whereas a *PDR1* gain of function mutant (GOF)^[1] shows better growth.

Upregulation of efflux transporter genes

- *Cgmip1Δ* constitutively overexpresses *PDR1*, and the efflux transporter genes *CDR1* and *CDR2*.

Cgmip1Δ shows a higher survival rate upon phagocytosis

- Phagocytosis survival assay upon exposure to human monocyte-derived macrophages: Only *Cgmip1Δ* and *PDR1* GOF mutants show higher survival rate at all times, whereas lack of *PDR1* leads to a lower survival rate.

Transient *petite* phenotype as a survival strategy

- Generation time of *C. glabrata* under different stresses: *C. glabrata* only adapts to FL and shows *petite* phenotype at the beginning of the long-term exposure experiment, which revert to respiratory metabolism.

Conclusions

- ❖ *Cgmip1Δ* is a *petite* mutant that shows resistance to FL and ER stress likely due to the upregulation of the transcriptional factor Pdr1 and the efflux pumps Cdr1 and Cdr2. This seems also to result in a better fitness upon phagocytosis, which will be confirmed in further experiments.
- ❖ The transient *petite* phenotype seems to be involved in the adaptation of *C. glabrata* to high concentrations of FL by switching between respiratory and fermentative metabolism, possibly by an epigenetically regulated loss of function of CgMIP1. Epigenetic studies will be performed.

References

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