Overview/Abstract

Infections due to *C. glabrata* are especially difficult to treat because this species is innately less susceptible to azoles, which are still the most commonly used antifungal drugs. Importantly, recent clinical reports suggest that azole antifungal resistance in *C. glabrata*, including multidrug resistance, is emerging at a rapid pace, thus posing an important challenge to therapeutic management[1, 2]. *C. glabrata* is more closely related to *Saccharomyces cerevisiae* than to *C. albicans* [3]. *C. glabrata,* can form "petite colonies" and grow without mtDNA [4, 5], and it can replicate within mononuclear phagocytes [6-8] because of its ability to modify the phagosomal pH [9, 10].

We were the first to show that *C. glabrata* undergo continuous asymmetric mitotic divisions in a process known as replicative aging, which was first defined in *S. cerevisiae* [11, 12]. Our work indicates that replicative aging is also relevant in a clonally expanding *C. glabrata* population as it allows the yeast cells to naturally change and attain resilience in the host environment. An exponentially growing yeast population is predominantly composed of young cells because doubling results in 50% virgin cells, and therefore a 15 generation (GEN) old cell is present only at a ratio of 1: 30.000, unless preferential killing of young cells occurs. Importantly, we have shown that such selection occurs during infection in the host. Our data indicate that older *C. glabrata* cells, accumulate *in vivo* during chronic infection in mice and humans [11, 13, 14]. We have shown that old *C. glabrata* cells manifest enhanced tolerance to antifungals. Transcriptome analysis of old and young *C. glabrata* cells indicated that genes of the ergosterol pathway and other genes associated with azole resistance were differentially regulated. Consistent with that observation, old *C. glabrata* cells were observed to be more tolerant to fluconazole (FLU) compared to young *C. glabrata* cells. Of particular interest is the fact that known azole efflux pumps were significantly upregulated in older cells. Why these transcriptional changes occur in older cells is not known. It is also not known to what extent accumulation contributes to heteroresistance in the fungal population. Standard antifungal testing is only performed on exponentially growing cultures, which are started from single colonies.

In this seed grant application, we intend to generate preliminary data for a multidisciplinary NIH grant to investigate how aging contributes to resistance in *C. glabrata* populations. Goal 1: We propose to clone a C. glabrata mutant strain that enriches old cells. This will overcome the barrier to isolating enough old cells for lipidomic and metabolomic studies. Goal 2: We generate preliminary data about the generational distribution and heteroresistance of *C. glabrata* populations that are collected through a patient cohort study. These data will determine the actual generational distribution of *C. glabrata* cells in a patient-derived fungal population through bud scar staining yeast cells in urine. At the same time, direct plating will inform us of the percentage of yeast cells resistant to azoles.

These data will be unique as they analyze the phenotype of a human-derived *C. glabrata* population that has not been grown in vitro. Most likely, replicative aging is a continuous natural process that occurs in all eukaryotic cells and is associated with significant phenotypic changes, which are reversible and epigenetically encoded. Knowledge gained through this seed grant will be valuable to writing a multi-disciplinary grant that examines the heterogeneity within a pathogen population.