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Seminar title: New Strategies In Fighting Drug Tolerance And Resistance In Yeast Infections

Abstract

The increasing emergence of drug-resistant fungal pathogens, together with the limited number of available antifungal drugs, present serious clinical challenges in treating systemic, life-threatening infections. Repurposing existing drugs to augment the antifungal activity of well-tolerated antifungals is a promising antifungal strategy with the potential to be implemented rapidly. Here, we explored three strategies to improve existing available drugs: study the antifungal drugs affect on genetics and morphology of yeast cells, directing azoles to their correct location and learning the mechanism by which colistin, a positively-charged antibacterial peptide, enhances the antifungal activity of fluconazole, the most widely used orally available antifungal.

First, we found three gens and different characteristics that are involve in trimera formation, as trimeras are the direct result of fluconazole treatment and seem to initiate aneuploidy and drug resistance. As trimeras are three cells attached together they share their cytoplasm and as a result, different cell cycle signals. In this thesis we approve that trimeras are forming by different cell cycle regulations defect. First defect starts in the START point of the cell cycle were the DNA replication is uncoupled with the morphology of the cells. *SOL1* checkpoint is playing major role in the starting point of trimera formation, its degradation indicating that the bud is big enough results in DNA replication. The lack of *SOL1* induces trimera formation without any drug exposure. Later on, more defects in the MEN also lead to trimera formation, mutants of ΔΔ*bub2* reduced dramatically the levels of trimeras in fluconazole and OE of DMA2 produced high levels of trimeras.

Second strategy in eliminating drug resistance and tolerance is to direct azoles to their correct location. For knowing localization of azoles we took two fluorescent azoles that were synthesized in prof. Mich Fridman's lab and tracked their location by fluorescent microscopy.

Both of the azoles accumulated in the mitochondria with high co-localization with MitoTrecker green staining. Study on *saccharomyces cerevisiae* reveal that the target of azoles- ERG11 synthesized in the ER, we assume that this occurs in *Candida albicans* as well. Thus, new fluorescent azole 7AC-azole was synthesized and by microscopy we approved that accumulate more in the ER. In different molecular methods we found that directing the azole to its correct location, reduce the MIC_{100} by >100 fold in various *candida albicans* strains.

In the last part I focused on reviling the mechanism by which fluconazole and COL synergizes. Colistin was specifically effective in reducing fluconazole tolerance, a subpopulation of cells that grow slowly in drug and that may promote the emergence of persistent infections and resistance, this was tested on a range of susceptible, drug-resistant isolates and species. Clinically relevant concentrations of colistin synergistically reduced fluconazole MIC_{50} by 4-fold (FICI 0.28). Mechanistically, colistin increased permeability to fluorescent azoles and to intracellular dyes, accompanied by an increase in cell death that was dependent upon inhibition of the ergosterol biosynthesis pathway either pharmacologically or genetically. The positive charge of colistin is critical to its antifungal, as well its antibacterial, activity and colistin directly binds several eukaryotic membrane lipids (L-α-Phosphatidylinositol ammonium salt, L-α-Phosphatidyl-L-serine and L-α-Phosphatidylethanolamine) that are enriched in the membranes of ergosterol-depleted cells. The results support the idea colistin binds to fungal membrane lipids, and permeabilize fungal cells in a manner that depends upon the degree of ergosterol depletion.