Glycomics & Lipidomics

Extended Abstract

Kinase mediated regulation of lipid metabolism and the crosstalk with drug tolerance mechanisms in Candida albicans

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Abstract

The occurrence of pathogenic fungal infections has been rising all over the world. Although the research is developing the field of mycology, there is still a need for the identification of reliable determinants of virulence and obtain successful therapy in human. Candida albicans synthesizes the major lipids component such as sterol and its membranes contain typically eukaryotic lipid species like phospholipids, phosphoglycerides, etc., which play important role in cellular processes such as membrane homeostasis, signaling, morphogenesis, cell wall integrity, etc. The incidences of Candida albicans cells acquiring multidrug resistant (MDR) are common, which in turn hamper their successful chemotherapy. In membrane of C. albicans changes in ergosterol composition by disruption of ERG genes results in improper surface localization of drug efflux pumps like Cdr1p. There are also instances where common regulation of MDR and lipid metabolism genes has been observed. MDR in C. albicans is closely linked to the status of membrane lipids like ergosterol. However mechanisms, which regulate these lipid changes at compositional level, are largely unknown. A recent study showed that several kinase defective mutants of C. albicans were susceptible to azoles which target ergosterol biosynthesis suggesting that these kinases somehow, either directly or indirectly, affect ergosterol biosynthesis pathway. In the present study we screened for the kinases whose knockouts might show an abrupt or depleted ergosterol levels. Based on thin layer chromatography and gas chromatography-mass spectrometry screens; we found some kinases, which showed defects in ergosterol biosynthesis. Interestingly, we found high ergosterol content in several kinase mutants, yet these were susceptible to azoles. Next we screened these select kinase mutants with drugs affecting different MDR pathways namely the cell wall and mitochondria. We further checked for the mycelia formation in these mutants. We have found some interesting correlation among these different pathways and based on our previous studies we hypothesize that ergosterol remains the key component of major MDR mechanism and that several kinases are involved in regulating the level of this fungal lipid. Though, the complete understanding of the ergosterol regulation via kinase circuitry requires further validation by correlation of molecular and lipidomic study, our study points towards a new functional role to these kinases in C. albicans.

Prolonged usage of antifungal azoles which target enzymes involved in lipid biosynthesis invariably leads to the development of multi-drug resistance (MDR) in Candida albicans. We had earlier shown that membrane lipids and their fluidity are closely linked to the MDR phenomenon. In one of our recent studies involving comparative lipidomics between azole susceptible (AS) and azole resistant (AR) matched pair clinical isolates of C. albicans, we could not see consistent differences in the lipid profiles of AS and AR strains because they came from different patients and so in this study, we have used genetically related variant recovered from the same patient collected over a period of 2-years. During this time, the levels of fluconazole (FLC) resistance of the strain increased by over 200-fold. By comparing the lipid profiles of select isolates, we were able to observe gradual and statistically significant changes in several lipid classes, particularly in plasma membrane microdomain specific lipids such as mannosylinositolphosphorylceramides and ergosterol, and in a mitochondrial specific phosphoglyceride, phosphatidyl glycerol. Superimposed with these quantitative and qualitative changes in the lipid profiles, were simultaneous changes at the molecular lipid species levels which again coincided with the development of resistance to FLC. Reverse transcriptase-PCR of the key genes of the lipid metabolism validated lipidomic picture. Taken together, this study illustrates how the gradual corrective changes in Candida lipidome correspond to the development of FLC tolerance. Our study also shows a first instance of the mitochondrial membrane dysfunction and defective cell wall (CW) in clinical AR isolates of C. albicans, and provides evidence of a cross-talk between mitochondrial lipid homeostasis, CW integrity and azole tolerance.

The phenomenon of Candida cells acquiring multidrug resistance (MDR) is common. This however hampers their successful chemotherapy. The Candida species infections by and large have been controlled by using the systemic azole drug fluconazole (FLC), and other topical azole and polyene drugs. The excessive use of FLC has however, resulted in the emergence of azole-resistant strains of Candida species. Candida albicans as well as non-albicans species have evolved a variety of mechanisms to develop MDR to common antifungals. Reduced intracellular accumulation of drugs (due to rapid efflux) is one of the most prominent mechanisms of resistance in Candida cells. Accordingly, clinical azole resistant isolates of C. albicans display transcriptional activation of genes, encoding ATP Binding Cassette (ABC) multidrug

transporter proteins CaCdr1p or CaCdr2p or Major Facilitator Super family (MFS) efflux pump protein CaMdr1p.

In C. albicans, lipids in addition to being the structural and metabolic components of yeast cells also play an important role in the frequently observed MDR. For example, CaCdr1p shows selectivity towards membrane recruitment and prefers membrane raft microdomains for its localization within plasma membrane. There are close interactions between raft constituents such as ergosterol and sphingolipids (SLs), and disruption of these results in altered drug susceptibilities. Thus, any change in ergosterol composition by disruption of ERG genes, or change in SL composition by disruption of its biosynthetic genes, leads to improper surface localization of CaCdr1p.

Interestingly, MFS transporter CaMdr1p shows no such selectivity towards raft lipid constituents and remains fully membrane localized and functional in cells where SL or ergosterol biosynthesis is compromised. There are also instances where common regulation of MDR and lipid metabolism genes have been observed. Changes in the status of membrane lipid phase (or membrane fluidity) and asymmetry also seem to affect azole resistance in C. albicans. Taken together, MDR in C. albicans is closely linked to the membrane lipid composition. The overall drug susceptibility of a cell appears to be an interplay of membrane lipid environment, drug diffusion and extrusion. Thus, it is quite apparent that lipids in some way or the other contribute to the development of resistant phenotype. However, the exact sets of changes that occur in the lipid composition leading to the resistant phenotype are not completely understood.

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