The role of *Candida albicans'* plasma membrane sphingolipids in the potentially new mechanism of drug resistance and the inflammatory response

Candida albicans are fungal opportunistic pathogens of humans, which in the presence of comorbidities can lead to death of patients. Since fungi and humans belong to *Eukaryotes*, there are a number of similarities in the structure and function of their cells, hence it is difficult to find antifungal drugs that would not harm human cells and kill the fungi.

Commonly used antifungal drugs target the ergosterol of cell membranes (polyene drugs, e.g. amphotericin B) or its biosynthetic pathway (azole drugs, e.g. fluconazole). Echinocandins (e.g. caspofungin), which damage the fungal cell wall, are also used. However, fungi have developed a number of defence mechanisms against drugs. In *C. albicans*, defence processes are known such as efflux drugs from the cell by membrane transporters and additional ergosterol synthesis. The increasing drug resistance of *C. albicans* forces researchers to make efforts to invent new antifungal structures, new strategies to combat mycoses, and learn about physiological processes that can help identify new drug targets.

Recently, attention has been paid to the interaction of sphingolipids with ergosterol in the membranes of a non-pathogenic fungus - baker's yeast (*Saccharomyces cerevisiae*). These two types of lipids arrange themselves in rigid structures called domains that ensure the correct localization of proteins, including transporters that efflux drugs from the cell. It appears that when the membrane is deficient in ergosterol by fluconazole treatment, yeast synthesizes more sphingolipids that can compensate for this loss of ergosterol. Research shows that the activity of some enzymes of the sphingolipid biosynthesis pathway also changes, which is why sphingolipids with a specific structure are synthesized.

However, little is known about the above processes in *C. albicans*, although there are already indications that this may be an as yet undiscovered mechanism of drug resistance.

In the proposed project, we want to study the interaction of sphingolipids with ergosterol in the cell membrane of *C. albicans* under the influence of azole drugs and amphotericin B. Based on the obtained results confirming the process of compensating ergosterol with sphingolipids, it will be possible to propose a new treatment strategy using a mixture of azoles with inhibitors of the sphingolipid biosynthesis pathway. We want to study the above tasks with the use of *C. albicans* with deletions of selected genes encoding of the enzymes of the sphingolipid and ergosterol biosynthetic pathway. Cultures will be carried out, the cell membrane isolated and the amounts of sphingolipids with a specific, altered structure will be analysed, as well as the biophysical parameters of plasma membrane in above mutants and in stress conditions will be measured.

Our research in recent months has shown that the loss of ergosterol in the membrane of *C. albicans* causes changes in the cell wall of this fungus, causing it to be unmasked from the host's immune system. In the proposed project, we would like to investigate how the defence of *C. albicans* against the loss of ergosterol, in which sphingolipids with a specific structure are synthesized, will affect the structure of the cell wall of this fungus and induce an inflammatory reaction in mammalian cells We want to use *C. albicans* mutant co-cultures with skin fibroblast cells and vaginal epithelial cells. We want to measure primarily the level of pro-and anti-inflammatory cytokines. We will also study changes in individual elements of the cell wall using quantitative fluorescence methods (FACS) and confocal as well as super resolution microscopy imaging.

Our project belongs to the field of basic research and aims to better understand the mechanisms of defence of *C. albicans* cells against known drugs, but the results may be important for practical applications. The discovery of a new mechanism of drug resistance could provide clues for finding ways to combat mycoses caused by this pathogen.